THE SYNTHESES OF NATURALLY OCCURRING NAPHTHAZARINS

A thesis presented for the degree of Doctor of Philosophy of the Australian National University

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To Edwin Charles Hughie Outram

Declaration

The work described in this thesis is original and has not been submitted for a degree or diploma at any other university or college. To my knowledge, it does not contain material previously published or presented by any other person except where due reference has been made in the text.

Felicity K. Moore

Acknowledgements

My most sincere thanks go to Dr Christina Chai for being an inspirational and challenging supervisor. Thank you very much for your determination, honesty and energy. Your efforts often extended above and beyond the call of duty and I will always be very appreciative of that. Thank you also to Professor Jack Elix for being so supportive- through both sage words of advice and the occasional provision of a glass of good red!

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Abstract

Over the past decades, many naturally occurring naphthoquinones have been shown to display biological behaviour. The synthesis of functionalised naphthoquinones, therefore, poses an interesting challenge to organic chemists. A brief review of the biological activity and the synthesis of polyfunctionalised naphthoquinones is presented in Chapter One.

A number of synthetic routes for the construction of the naphthoquinone bicyclic core have been developed previously. We utilised two [4+2] Diels-Alder cycloaddition approaches reported by Brassard *et al.*, as well as the Claisen condensation/oxidation methodology of Bycroft *et al.*, to synthesise the key precursor, 2,5,7-trimethoxy-1,4-naphthoquinone. This naphthoquinone was then used as a platform for further synthetic studies, culminating in the total synthesis of the naturally occurring naphthazarins 2,5,7,8-tetrahydroxy-3,6-dimethyl-1,4-naphthoquinone (aureoquinone), 3-ethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone and 3-ethyl-2,5,7,8-tetrahydroxy-6-methyl-1,4-naphthoquinone. The structure of the latter was confirmed to be identical to that of boryquinone, through chromatographic and spectroscopic comparison of the synthetic compound and the natural product isolated from the lichen *Cladonia boryi* Tuck. These synthetic studies are described in Chapter Two.

In 1999, the novel *bis*-naphthazarin derivative hybocarpone was isolated from the lichen *Leconora hybocarpa* (Tuck.) Brodo by Elix *et al.* during routine screening of lichen secondary metabolites. Chapter Three describes the formal total synthesis of hybocarpone utilising the Claisen condensation/oxidation approach to the construction of the bicyclic framework.

The dimerisation of the monomeric naphthazarins was also investigated, with a view to developing an expedient total synthesis of hybocarpone and naturally occurring structural analogues. In ensuing studies, two novel dimers of the naturally occurring naphthoquinone phthiocol were synthesised and their structures were elucidated by X-ray crystallographic analysis. This work is described in Chapter Four.

Given the established bioactivity of many naturally occurring naphthoquinones we then undertook some preliminary biological studies on the compounds prepared above and

this work is described in Chapter Five. The antiproliferative activity of aureoquinone, boryquinone, hybocarpone and menadione (Vitamin K_3) against HeLa cells was evaluated via an MTT assay. The monomeric naphthazarins and menadione displayed weak to moderate cytotoxicity in this assay and hybocarpone was shown to possess activity in the micromolar range.

The procedural details for all experiments conducted are given in Chapter Six, along with the relevant data used to characterise the synthetic compounds.

Abbreviations

The following abbreviations have been used within this thesis:

Ac	acetyl
Anal.	Analysis
aq.	aqueous
b.p.	boiling point
Bn	benzyl
Bu	butyl
ca.	*circa* (approximately)
CAN	ceric ammonium nitrate
Calcd	Calculated
δ	Chemical shift (parts per million)
decomp.	decomposed
DMF	*N*, *N*-dimethylformamide
EDTA	ethylenediaminetetraacetic acid
e.g.	*exempli gratia* (for example)
EI	Electron Impact
et al.	*et alii* (and others)
Et	Ethyl
h	hour
HR	High Resolution
Hz	Hertz
i.e.	*id est* (that is)
IR	infrared
LDA	Lithium Diisopropylamine
Lit.	Literature

LR	Low Resolution
m	*meta*
m-CPBA	*m*-Chloroperbenzoic Acid
Me	Methyl
MHz	Mega Hertz
min	minute(s)
MMT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
mol	mole(s)
Mol.	Molecular
m.p.	melting point
MS	Mass Spectroscopy
m/z	Mass to charge ratio
NMR	Nuclear Magnetic Resonance
o	*ortho*
p	*para*
PBS	Phosphate Buffered Saline
Ph	Phenyl
ppm	parts per million
Pr	Propyl
R	alkyl group
rpm	revolutions per minute
R_f	Retardation factor
SAR	Structure Activity Relationship
TBAB	Tetrabutylammonium bromide
THF	tetrahydrofuran
TLC	Thin Layer Chromatography

TMEDA	Tetramethylethylenediamine
TMS	Trimethylsilyl
UV	Ultraviolet
Wt.	Weight

Publications and Presentations

Some of the work described in this thesis has been reported and presented in the following publication and presentations.

Chai, Christina L. L.; Elix, John A.; Moore, Felicity K. E.; An expedient and efficient synthetic route to some naturally occurring polyfunctional naphthazarins, *Tetrahedron Lett.* **2001**, 42, 8915-8917.

Towards the Synthesis of Naturally Occurring Naphthoquinones, poster presented at the World Chemistry Congress, Brisbane, Australia, 2001.

Towards the Synthesis of Naturally Occurring Bis-*Naphthazarin Derivatives*, poster presented at the 14th International Conference on Organic Synthesis, Christchurch, New Zealand, 2002.

Towards the Synthesis of Naturally Occurring Bis-*Naphthazarin Derivatives*, Oral Presentation at 10th Asian Chemical Congress/ 8th Eurasian Conference on Chemical Sciences, Hanoi, Vietnam, 2003.

Chapter One: Introduction

1.1 Introduction

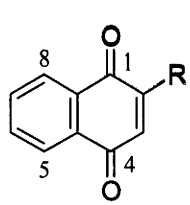
The World Health Organisation estimates that a quarter of modern medicines were first used in traditional remedies derived from plants.[1] The link between modern science and culturally derived therapeutics is well established and has directed many drug discovery programmes. Consequently, the role of natural products in global efforts to combat disease and infection is widely recognised.[2,3]

More particularly, naturally occurring quinones have been of medicinal interest for decades due to the bioactivity displayed by a number of lead compounds. Of the quinonoid natural products, those containing the naphthoquinone structural motif constitute the largest sub-class, as approximately four hundred naturally occurring naphthoquinones have been isolated and characterised to date.[4]

The ethnopharmacological history of naphthoquinones reveals that plant extracts containing these compounds have been used as hair and skin dyes and, more importantly, in herbal medicines for thousands of years.[5] Naphthoquinones are of commercial value today due to their use as colouring agents in the dye industry, pesticides, anti-cancer drugs and as the active components in anti-inflammatory preparations.[6]

1.1.1 Naturally Occurring 1,4-Naphthoquinones

The parent 1,4-naphthoquinone (**1.1**) has been identified in the defensive secretion of the spider *Phalangium opilio*, commonly known as the Daddy Longlegs or Harvestman spider, as well as in the leaves of various higher plants. The herbicidal activity of 1,4-naphthoquinone (**1.1**) has been recently established and shown to be due to the inhibition of a plant enzyme involved in the biosynthesis of carotenoids (IC_{50} value* 4 μM).[7]



1.1 R = H, 1,4-Naphthoquinone

1.2 R = Me, Menadione

Figure 1.1 Parent 1,4-naphthoquinone (1.1) and menadione (1.2)

* In general terms, an IC_{50} value refers to the concentration of a given compound required to elicit fifty per cent of a measurable response.

Menadione (Vitamin K_3, **1.2**) has been isolated from the ferns *Asplenium laciniatum* and *A. indicum* (Polypodiaceae). The bioactivity of menadione (**1.2**) has been studied extensively, as menadione (**1.2**) is known to undergo redox cycling to generate reactive oxygen species and thereby cause oxidative cellular damage.[8] Menadione (**1.2**) has been shown to induce both apoptosis and necrosis in rat pancreatic acinar AR4-2J cells[9] and to induce single and double strand DNA breaks in human MCF-7 cells through a process initiated by the generation of free radicals.[10]

1.1.2 Naturally Occurring Hydroxy-1,4-Naphthoquinones

The naphthoquinone core of many naturally occurring compounds is oxygenated to some extent, and hydroxy-1,4-naphthoquinones generally exhibit more pronounced biological activity than non-oxygenated analogues. To date, the structure-activity relationship (SAR) for hydroxy-1,4-naphthoquinones has not been fully elucidated. The activity trends observed within this class of compound are very dependent on the nature of the biological assay performed. For example, while some investigations have indicated that C5 hydroxylation prescribes anti-tumour promoting effects on short term Epstein-Barr early antigen activation,[11] herbicidal activity[12] and toxicity to rat hepatocytes,[13] others suggest that a C2 hydroxy functionality is responsible for herbicidal and insecticidal activity.[14,15] It is evident, nonetheless, that hydroxy-1,4-naphthoquinones constitute an important class of bioactive compounds (Figure 1.2).

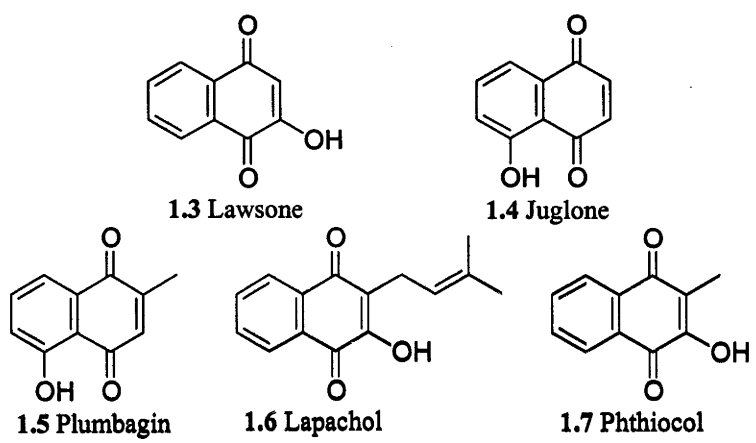


Figure 1.2 Naturally occurring hydroxy-naphthoquinones

Over four thousand years ago, the ancient Egyptians used the leaves of the North African shrub *Lawsonia inermis*, commonly referred to as the Henna tree, to dye their hair red.[16] The colouring component is 2-hydroxy-1,4-naphthoquinone (lawsone, **1.3**) which can also be extracted in significant quantities from the seeds of the plant *Lomatia*

ferruginea (Proteaceae). Henna can be used to effectively dye both hair and skin and has developed significant cultural and religious value throughout Africa, the Middle East and India (Figure 1.3).[17] Lawsone (**1.3**) also displays antipruritic effects on scratching behaviour in mice.[18]

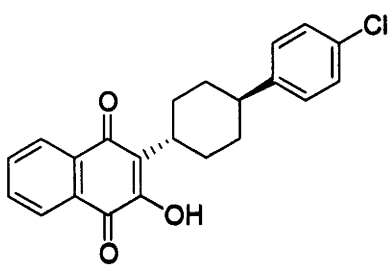
Figure 1.3 Henna-dyed hands at an Indian wedding ceremony (Photos courtesy of Siddhartha Rao)

Juglone (**1.4**), a structural isomer of lawsone (**1.3**), and the *C*-methyl analogue plumbagin (**1.5**) are ubiquitous yellow pigments found in *Juglans regia* (Black Walnut), the leaves and nuts of various other plants, as well as in fungal cultures (Figure 1.2).[19,20] Preparations of *Juglans regia* have been used traditionally as dyes and are still applied topically for the treatment of inflammatory skin diseases, including fungal, bacterial and viral infections, ringworm and acne.[4] Juglone (**1.4**) displays cytotoxicity against murine lymphosarcoma L5178Y cells.[21]

Plumbagin (**1.5**) has been isolated from bark samples of *Diospyros maritima* collected throughout South-East Asia. Extracts of this plant are used extensively in topical skin treatments in Indonesia and for the treatment of rheumatic diseases in Taiwan.[22,23] The plant *Plumbago zeylanica* (Plumbaginaceae), traditionally known as 'Bach hoa xa', also

contains high levels of plumbagin (**1.5**) and is used in Vietnamese medicine for the treatment of numerous skin conditions, rheumatic pain and even cancer.[24] Plumbagin (**1.5**) has also been isolated from the aerial parts of *Plumbago pearsoni* and from the roots, bark, wood and flowers of numerous plant species.[4] Plumbagin (**1.5**) has been recently found to display potent anti-malarial, anti-microbial and anti-cancer activity.[25] Various studies into the pesticidal activity of plumbagin (**1.5**) have also been reported, and the molluscicidal activity against the snail *Biomphalaria glabrate* was found to be significant.[26] This organism presents a serious risk of schistosomiasis, a parasitic infection contracted through insanitary water.[27]

Lapachol (**1.6**) is a structurally related naphthoquinone that has been detected in the wood, bark and roots of many plants of the family Bignoniaceae.[28] The potassium salt of lapachol (**1.6**) also displays significant molluscicidal activity against *Biomphalaria glabrate*.[27] *Tabebuia* tree extracts containing lapachol (**1.6**) known as 'pau d'arco', have been used in folk medicine by Maka and Callawaya Indians to cure a number of diseases, including cancer.[29] However, the National Cancer Institute in the United States has subsequently identified a number of undesirable side effects, such as vomiting and nausea, which have precluded the use of lapachol (**1.6**) in modern chemotherapeutic treatments.[4,30] Lapachol (**1.6**) also displays significant anti-malarial activity.[31] Investigations into the biological properties of lapachol (**1.6**) analogues led to the development of atovaquone (**1.8**), which is currently prescribed for the treatment of pneumonia (Figure 1.4).[4]



1.8

Figure 1.4 Anti-pneumonia drug atovaquone (1.8)

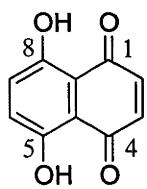
Phthiocol (**1.7**), a structural isomer of plumbagin (**1.5**), is produced by *Mycobacterium tuberculosis* and has been shown to generate superoxide radicals *in vitro* and to inhibit cell growth.[32] It is thought that phthiocol (**1.7**) may be involved in the biological mechanism leading to the pathogenicity of *Mycobacterium tuberculosis* and that an

understanding of this pathway could lead to the development of new anti-tuberculosis agents.[33]

The bioactivity of these hydroxy-1,4-naphthoquinones continues to be investigated and it is anticipated that comprehensive SAR studies will be forthcoming. Established folklore, combined with the bioavailability of these compounds, however, means that they are widely considered to be attractive medicinal agents, particularly in less developed countries.

1.1.3 Naturally Occurring Naphthazarins

Naturally occurring naphthazarins contain a 5,8-dihydroxy-1,4-naphthoquinone structural core and constitute a particularly active class of compounds, with many having been shown to display antibiotic and anti-oxidant activity.[34,35] The parent naphthazarin (**1.9**) has been used historically as a purple dye and was isolated from the wood and bark of *Lomatia obliqua* (Proteaceae) and from the walnut husks of *Juglans mandschurica maxim* var. *sieboldiana* (Figure 1.5).[36] The toxicity of naphthazarin (**1.9**) to the fish *Oryzias latipes* is due to the inhibition of mitochondrial oxidative phosphorylation pathways.[37]



1.9

*Figure 1.5 Naphthazarin (**1.9**)*

Several bioactive naphthazarins have recently been isolated and characterised.[38,39] One important group of naphthazarin natural products contain a spiro ring system appended to the naphthazarin core. Fredericamycin A (**1.10**), for example, is a structurally complex natural product isolated from *Streptomyces griseus*, which contains six ring systems with a central spiro moiety (Figure 1.6). The bioactivity of fredericamycin A (**1.10**) has been investigated and its potency against bacterial and cancer cells and the inhibition of both RNA synthesis and topoisomerase I and II has been established.[40]

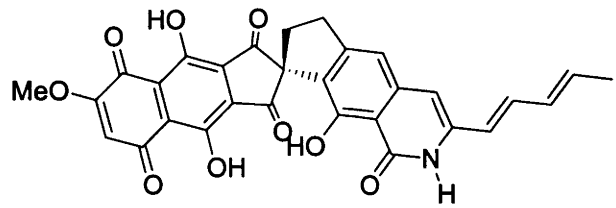


*Figure 1.6 Fredericamycin A (**1.10**)*

γ-Rubromycin (**1.11**), purpuromycin (**1.12**) and heliquinomycin (**1.13**) are structurally related natural products containing the naphthazarin moiety linked via a spiroketal ring system to an isocoumarin fragment (Figure 1.7). These derivatives are known as the 'griseorhodin' class of compounds and have been shown to display potent antibiotic and anti-tumour activity.[41] γ-Rubromycin (**1.11**) displays activity against the reverse transcriptase of HIV and human telomerase, an enzyme that is overproduced in cancer cells. Purpuromycin (**1.12**) is used as a topical agent for bacterial infections and heliquinomycin (**1.13**) displays activity as an inhibitor of DNA helicase, an enzyme involved in DNA replication and repair.[42,43]

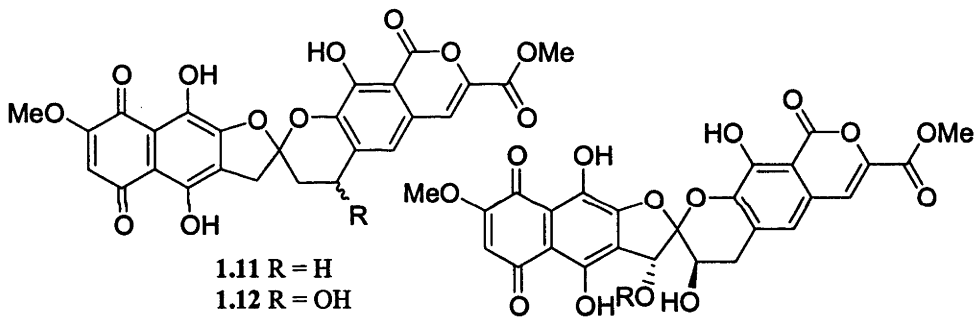
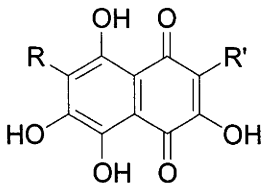


Figure 1.7 The Griseorhodin Naphthazarins

Berg *et al.* have recently isolated the fungal metabolite aureoquinone (**1.14**) from surface cultures of an *Aureobasidium* sp. grown on a synthetic medium.[44] Aureoquinone (**1.14**) displays moderate anti-microbial activity and inhibits a number of different proteases. Moore and co-workers have previously isolated the structurally related 3-ethyl-2,7-dihydroxynaphthazarin (**1.15**) during degradation studies of the echinoid pigment, spinochrome A (Figure 1.8).[45]



1.14 R = R' = Me Aureoquinone
1.15 R = H, R' = Et
1.16 R = Me, R' = Et Boryquinone?

Figure 1.8 Naturally occurring 2,7-dihydroxynaphthazarins

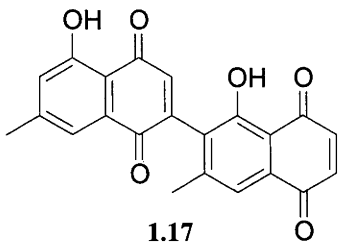
Huneck *et al.* have reported the isolation of 'boryquinone', a lichen metabolite isolated from cultures of *Cladonia boryi* (Tuck.) Cladoniaceae.[46] The authors did not propose a structure for this compound but the spectral data reported are consistent with the naphthazarin structure **1.16** (Figure 1.8). The biological activities of naphthazarin **1.15** and boryquinone have not been evaluated but would be of interest due to the promising bioactivity reported for aureoquinone (**1.14**). Structurally related naphthazarins have been isolated recently from the mycobiont cultures of the lichen *Cladonia cristatella.*[47] In particular, the 7-*O*-methyl derivatives of naphthazarins **1.15** and **1.16** were isolated and characterised.

1.2 Naturally Occurring Naphthoquinone Dimers

As the naphthoquinone structural motif has been widely recognised as a pharmacophore in medicinal chemistry, the isolation of naturally occurring dimeric naphthoquinones has generated considerable interest.[48-64]

1.2.1 Naturally Occurring Bis-*Naphthoquinones*

Species of the plant genus *Diospyros* are found throughout Africa and South-East Asia and are renowned for the production of naphthoquinones in the bark and wood, particularly *bis*-naphthoquinones.[65-70] Diospyrin (**1.17**) is a dimeric naphthoquinone[68] which has been isolated from *D. abyssinica*, *D. fragrans*, *D. kamerunensis* and *D. longiflora*. Diospyrin (**1.17**) exhibits anti-tumour, antibacterial and anti-leishmanial activity.[71,72] Many structural isomers, including elliptinone (**1.18**), have also been isolated from the genus *Diospyros* (Figure 1.9).



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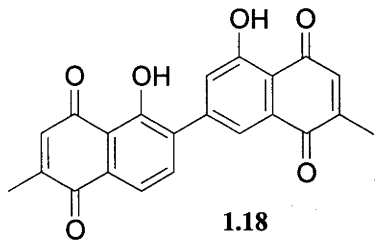


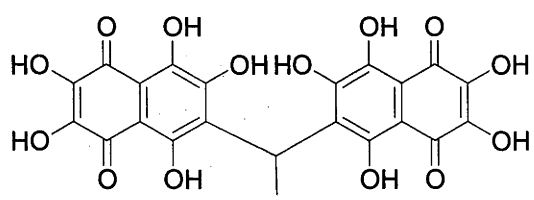
1.18

Figure 1.9 Bis-*naphthoquinones diospyrin* ***(1.17)*** *and elliptinone* ***(1.18)***

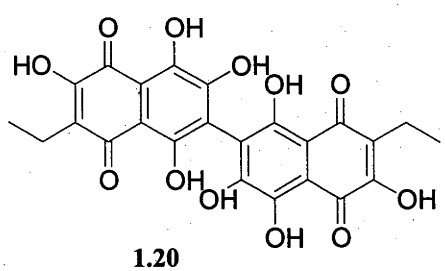
Elliptinone (**1.18**) and analogue dimers display weak anti-tumour activity, but they are significantly less active than the corresponding monomeric derivatives which have been tested.[11,73]

1.2.2 Naturally Occurring Bis-*Naphthazarin Derivatives*

Dimers containing the naphthazarin structural motif have also been isolated and characterised. For example, the dimeric naphthazarin **1.19** was isolated from the sea urchin *Strongylocentrotus intermedius* and contains an alkyl linkage between the naphthazarin moieties. The structurally related 6,6'-*bis*-(3-ethyl-2,7-dihydroxynaphthazarin) (**1.20**) has been isolated by Thomson *et al.* from the deep sea holothuroids *Psychopotes longicauda* (Figure 1.10).



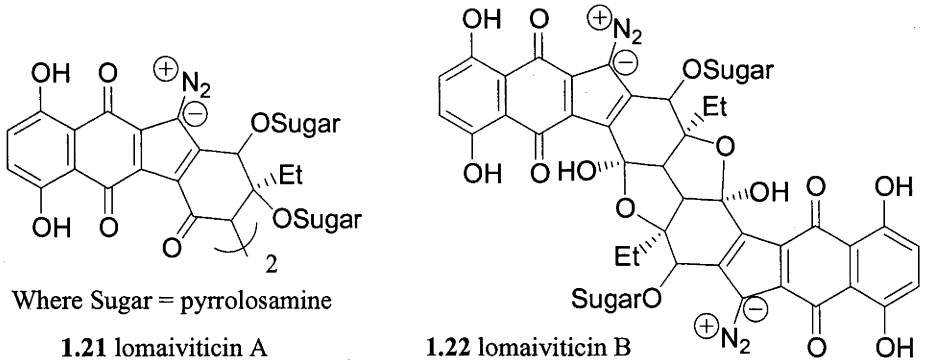
1.19



1.20

Figure 1.10 Bis-*naphthazarins* ***1.19*** *and* ***1.20***

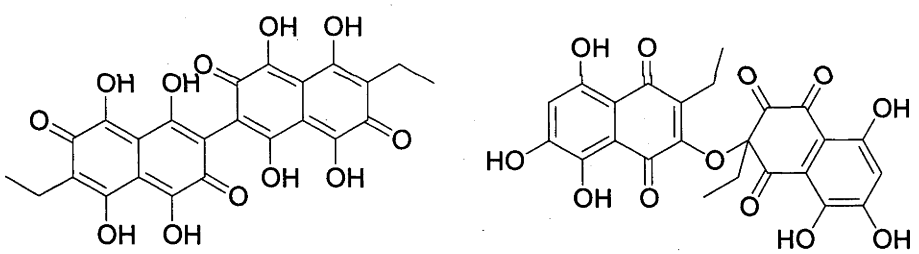
Lomaiviticins A and B (**1.21** and **1.22**) were recently isolated from the symbiotic actinomycetes associated with the marine ascidian *Polysyncraton lithostrotum* (Figure 1.11). The lomaiviticins comprise a complex dimeric structure containing the naphthazarin motif, and have been found to display potent DNA damaging activity against a range of cancer cell lines.[74]



1.21 lomaiviticin A **1.22** lomaiviticin B

*Figure 1.11 The lomaiviticins A and B (**1.21** and **1.22**)*

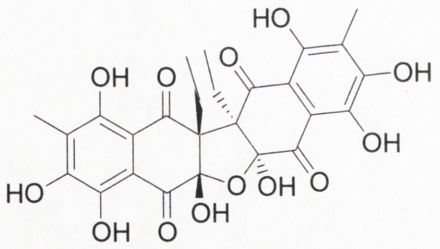
Dimeric naphthazarins have also been isolated from lichen sources.[75,76] The first reported lichen-derived *bis*-naphthoquinone was cuculuquinone (**1.23**), isolated from *Flavocetraria cucullata* [≡ *Cetraria cucullata*]. The authors assigned an unusual bis*amphi*quinone structure to derivative **1.23** based on its behaviour in acidic solutions. These authors also isolated islandoquinone (**1.24**) from the lichen *Cetraria islandica* var. *polaris*, and established its structure through proton NMR spectroscopy (Figure 1.12). The known marine natural product, *bis*-naphthazarin **1.20** was also isolated during the course of their investigation (Figure 1.10).[77]



1.23 **1.24**

*Figure 1.12 Lichen derived natural products cuculuquinone (**1.23**) and islandoquinone (**1.24**)*

During routine screening of the secondary metabolites from lichens, Elix *et al.* isolated the optically active lichen metabolite hybocarpone (**1.25**) from the mycobiont cultures of *Leconara hybocarpa* (Tuck.) Brodo.[78] Proton and carbon NMR spectroscopic techniques facilitated the structural elucidation of hybocarpone (**1.25**) and X-ray crystallography was then used to confirm this structure and establish the relative stereochemistry of the four contiguous stereocentres. Hybocarpone (**1.25**) has unique molecular architecture, as it consists of a symmetrical dimer of naphthazarin derivatives bridged by a hemiacetal linkage.



Only relative stereochemistry is known.

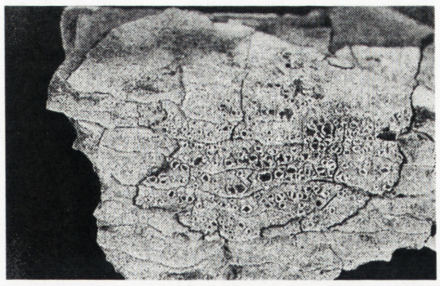


*Figure 1.13 Hybocarpone (**1.25**) and* Leconora hybocarpa *collected from woodland in Louisiana, United States of America by Dr Hamada*[*]

Hybocarpone (**1.25**) was shown to display cytotoxic activity against the P815 mastocytoma cancer cell line (IC_{50} value 0.27 μM). The bioactivity of the majority of naturally occurring *bis*-naphthoquinones and *bis*-naphthazarins, however, has not been evaluated to date. An investigation into the relative biological activity of monomeric naphthoquinones and the corresponding dimeric analogues would therefore provide a valuable SAR study.

1.3 General Synthetic Approaches to 1,4-Naphthoquinones and the Corresponding Dimers

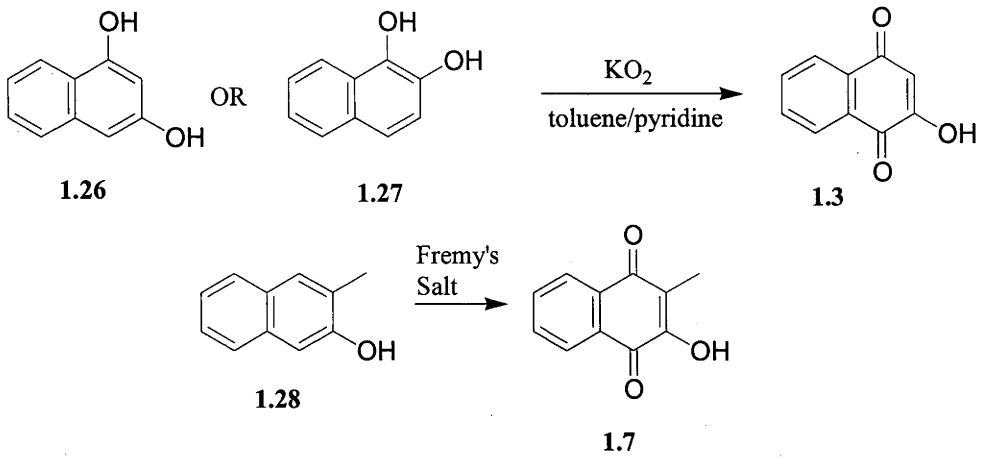
Given the number of naturally occurring bioactive naphthoquinones that have been isolated, general synthetic routes to polyfunctionalised naphthoquinones are in great demand.[79] Synthetic approaches to naphthoquinones have usually involved either the structural elaboration of very simple, readily available naphthalene or naphthoquinone precursors, or the construction of the bicyclic framework utilising powerful carbon-carbon bond forming reactions, with subsequent structural elaboration.

1.3.1 The Synthesis of Functionalised Naphthoquinones via the Structural Elaboration of a Pre-existing Bicyclic Framework

Many syntheses of naphthoquinones reported previously proceed via the oxidation of the appropriately substituted naphthol precursors, and suitable oxidative procedures are continually being developed for this purpose.[80-83] A number of bioactive hydroxy-1,4-naphthoquinones have been accessed through the application of this methodology. For

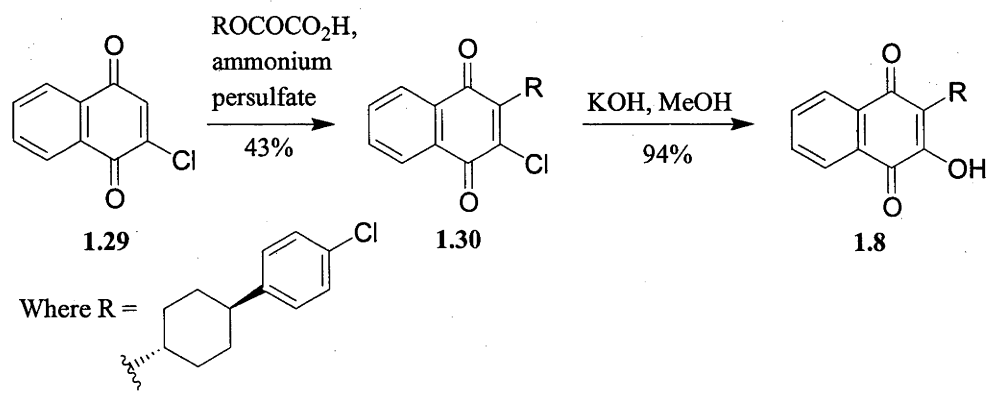
[*] A voucher specimen identified by Dr H. T. Lumdsch (Essen, Germany), is deposited at the Institute of Public Health and Environmental Sciences, Osaka City, Japan (NH 95112689).

example, Oliveros and co-workers have recently reported the use of potassium superoxide to convert 1,3-dihydroxynaphthalene (**1.26**) or 1,2-dihydroxynaphthalene (**1.27**) into lawsone (**1.3**).[84] The synthesis of phthiocol (**1.7**) has also been achieved by this approach, via the oxidation of naphthol **1.28** using Fremy's salt (Scheme 1.1).[85]



Scheme 1.1 Naphthol oxidation approaches to lawsone (1.3) and phthiocol (1.7)

The structural elaboration of simple naphthoquinones has also enabled the synthesis of functionalised naphthoquinones.[86-88] This general approach has been applied successfully to the synthesis of atovaquone (**1.8**). Radical mediated alkylation reactions were used to convert readily available 2-chloro-1,4-naphthoquinone (**1.29**) to the naphthoquinone derivative **1.30** in moderate yield. Subsequent nucleophilic displacement of the chloro group by treatment with methanolic potassium hydroxide gave rise to atovaquone (**1.8**) (Scheme 1.2).[89]



Scheme 1.2 Williams et al. *synthesis of atovaquone (1.8)*

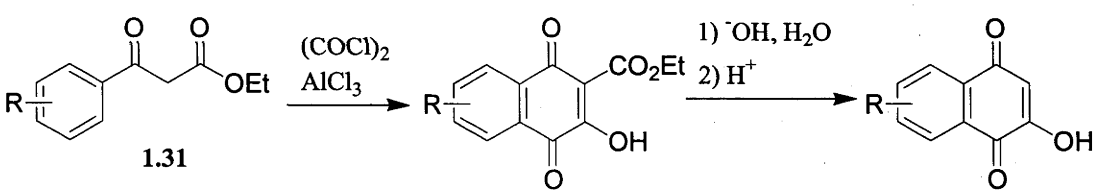
The synthesis of structurally complex naphthoquinones from simple naphthalenes and naphthoquinones can be somewhat limited, however, by the regioselectivity and reactivity requirements of structural elaboration. Alternative routes to highly

functionalised naphthoquinones are therefore synthetically attractive and several key protocols have been established.

1.3.2 The Synthesis of Functionalised Naphthoquinones via the de novo *Construction of the Bicyclic Framework*

Many naphthoquinone syntheses have involved the *de novo* construction of the bicyclic framework, as such an approach can facilitate the assembly of structurally complex molecules.

The Friedel-Crafts acylation reaction has been used to assemble highly functionalised aromatic systems and can also be utilised in the synthesis of 1,4-naphthoquinones. This is illustrated in Scheme 1.3, as the treatment of β-keto esters such as **1.31** with oxalyl chloride in the presence of a Lewis acid results in the formation of hydroxy naphthoquinones **1.32**.[90] Subsequent hydrolysis and decarboxylation of naphthoquinone **1.32** can be readily achieved via treatment with aqueous base to give the corresponding hydroxy naphthoquinone derivatives **1.33** (Scheme 1.3).



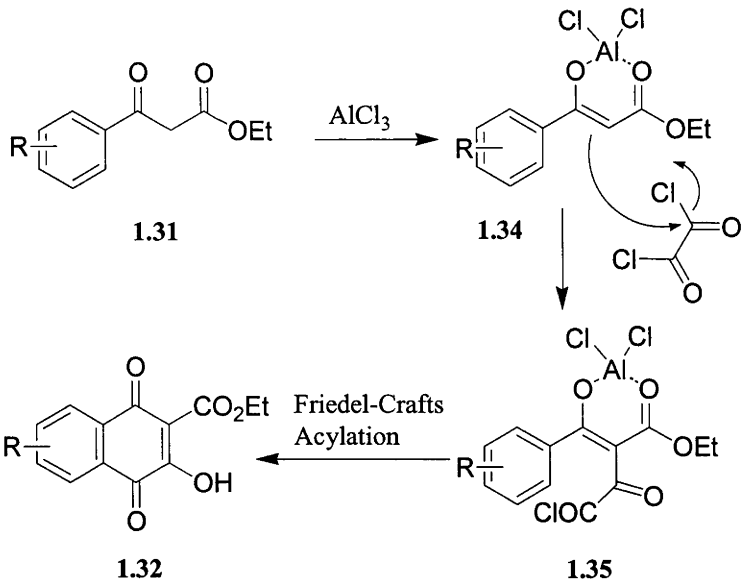
1.31 Where R = H, Me, OMe, Cl

1.32

1.33

Scheme 1.3 Friedel-Crafts cyclisation approach to naphthoquinones

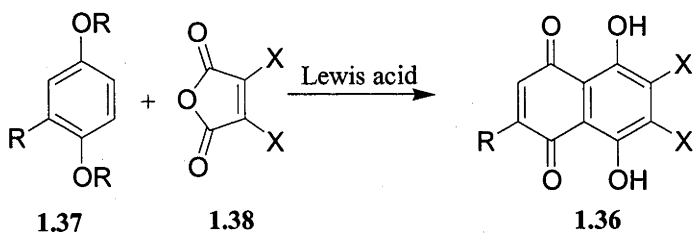
The mechanism proposed by the authors for the formation of the naphthoquinone framework via this approach is depicted in Scheme 1.4. Lewis acid coordination of aluminium trichloride to the enol tautomer of β-keto ester **1.31** gives rise to the intermediate derivative **1.34**. The nucleophilic addition of derivative **1.34** to oxalyl chloride affords the acyl chloride **1.35**, which then undergoes an intramolecular Friedel-Crafts acylation to give the hydroxy naphthoquinone **1.32**. Various hydroxy naphthoquinones have been synthesised in this manner in good to excellent yields.[90]



*Scheme 1.4 Postulated mechanism for the formation of naphthoquinone **1.32***

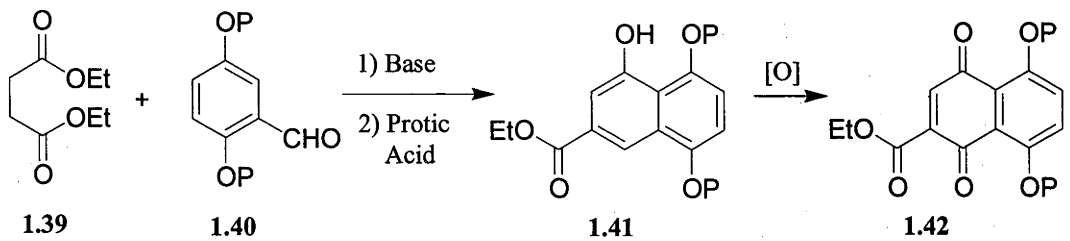
The construction of a functionalised naphthoquinone core has also been achieved via the intermolecular Friedel-Crafts acylation of an *O*-alkylated aromatic system with a maleic anhydride derivative.[91] This can be illustrated by the synthesis of the naphthazarin nucleus of type **1.36** following the treatment of 1,4-dialkoxybenzenes **1.37** with a maleic anhydride derivative **1.38** in the presence of a Lewis acid catalyst. The alkyl ethers are presumably cleaved *in situ* by the Lewis acid and aerial oxidation gives rise to the naphthazarin derivative **1.36** (Scheme 1.5).

This route has been utilised for the synthesis of several naphthazarins. The yields observed for this route are greatly increased when chloro substituted maleic anhydride derivatives are employed (Scheme 1.5).[92-94]



*Scheme 1.5 The synthesis of a naphthazarin of type **1.36***

The use of maleic anhydride as the electrophilic carbon equivalent enables the introduction of a four-carbon unit to construct the naphthoquinone core. The Stobbe condensation approach similarly involves the reaction of a 1,4-dicarbonyl unit with an aromatic substrate. Diethyl succinate (**1.39**) reacts with aromatic aldehydes **1.40** under basic conditions to give naphthol derivatives such as **1.41**. The subsequent oxidation of the naphthol **1.41** can then give rise to the corresponding 1,4-naphthoquinone **1.42** (Scheme 1.6). The synthesis of a variety of naphthoquinone derivatives has been achieved utilising this methodology.[95]

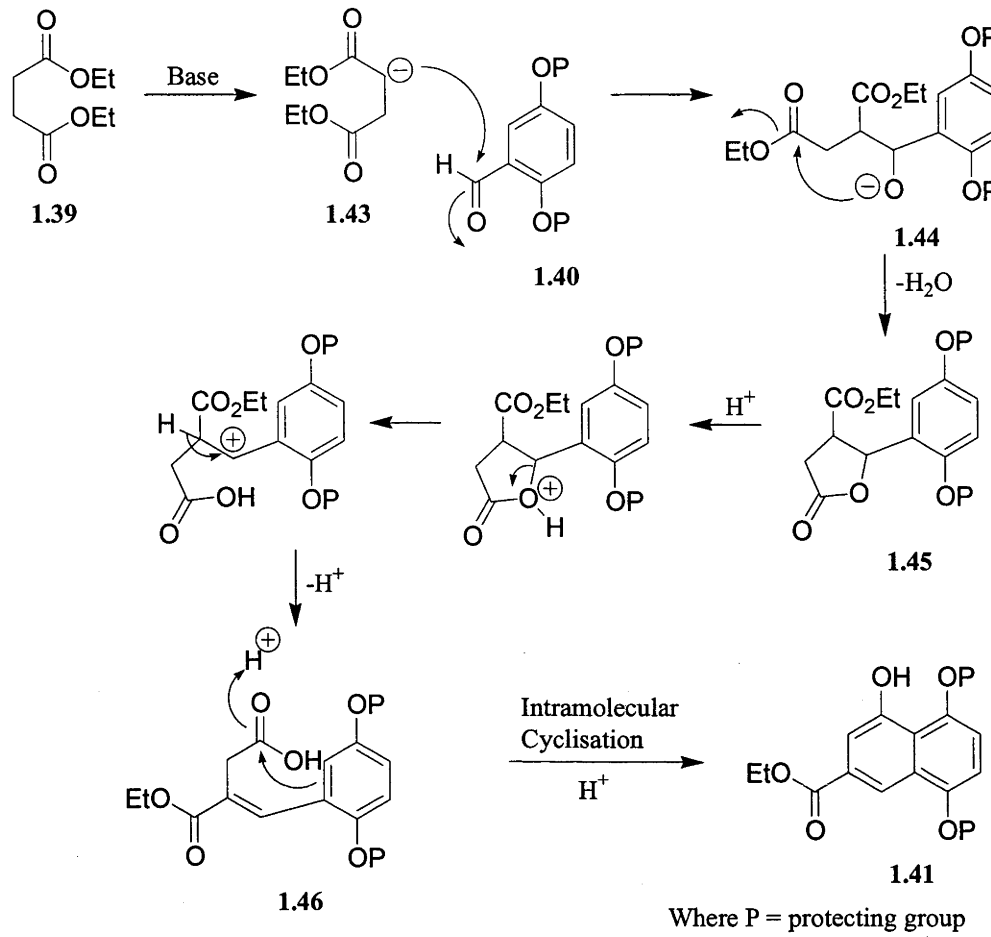


1.39 **1.40** **1.41** **1.42**

Where P = protecting group

Scheme 1.6 Stobbe condensation approach to naphthoquinone construction

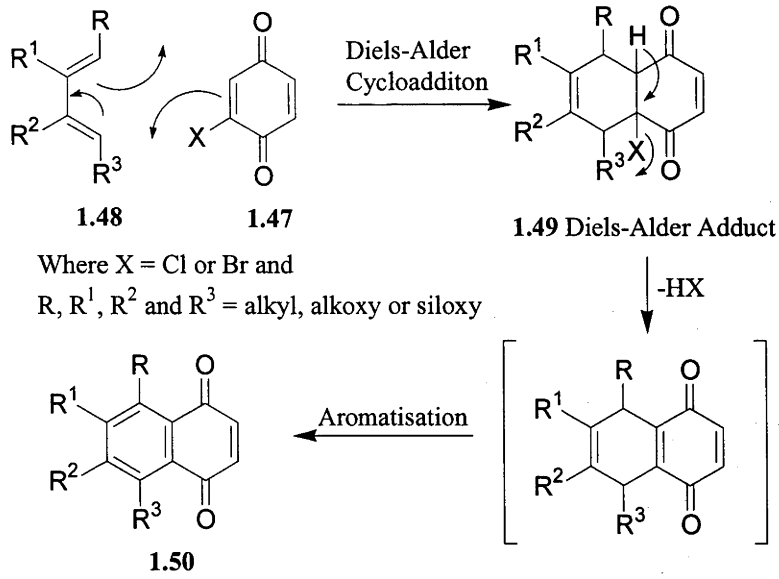
The construction of the naphthoquinone framework through this approach is thought to proceed via the mechanism depicted in Scheme 1.7. The anion **1.43** generated through treatment of diethyl succinate (**1.39**) with base reacts with the benzaldehyde **1.40** to afford the oxy-anion **1.44**. Intramolecular nucleophilic attack of the alkoxide then gives rise to an intermediate cyclic lactone **1.45**, which ring opens to give the carboxylic acid **1.46** after the addition of a strong protic acid. Acid catalysed cyclisation then results in the formation of the bicyclic structure and naphthol **1.41** is formed following aromatisation.



Scheme 1.7 Proposed mechanism for the formation of the bicyclic framework via the Stobbe condensation approach

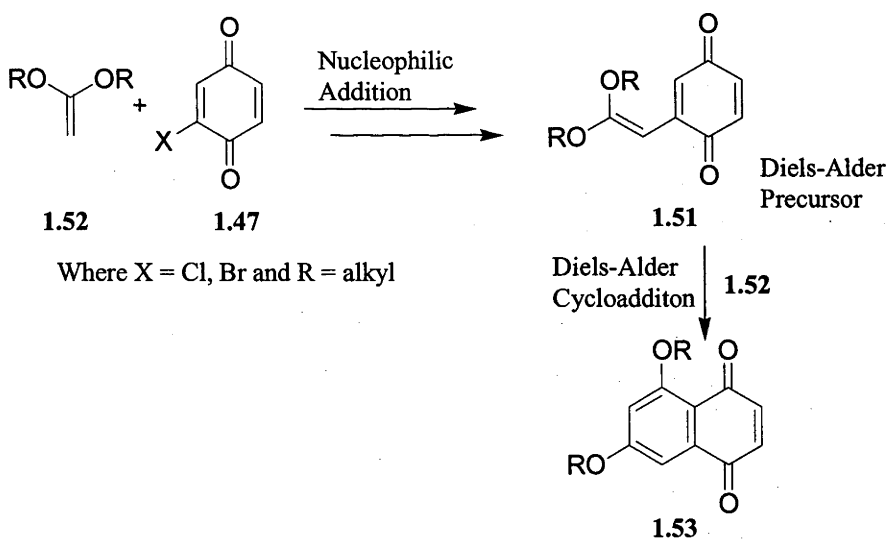
Various other approaches to the synthesis of the bicyclic naphthoquinone framework have been reported recently, including the use of a Michael-addition reaction,[96,97] the Dotz annulation of chromium carbenes[98,99] and the ring expansion of cyclobutene derivatives.[100] To date, however, none of these methods surpass the [4+2] Diels-Alder cycloaddition approach, in terms of synthetic utility.[101-103] The Diels-Alder reaction has been frequently used for the construction of the naphthoquinone framework, as the electronic factors governing reactivity and regioselectivity are generally well understood.

Halogenated benzoquinones **1.47** are commonly employed as dienophiles as they readily undergo cycloaddition reactions with butadiene derivatives **1.48** to give the corresponding Diels-Alder adducts **1.49**. The elimination of HX, followed by aromatisation can then lead to the formation of the 1,4-naphthoquinone **1.50** (Scheme 1.8).



Scheme 1.8 Diels-Alder cycloaddition of butadienes with benzoquinones

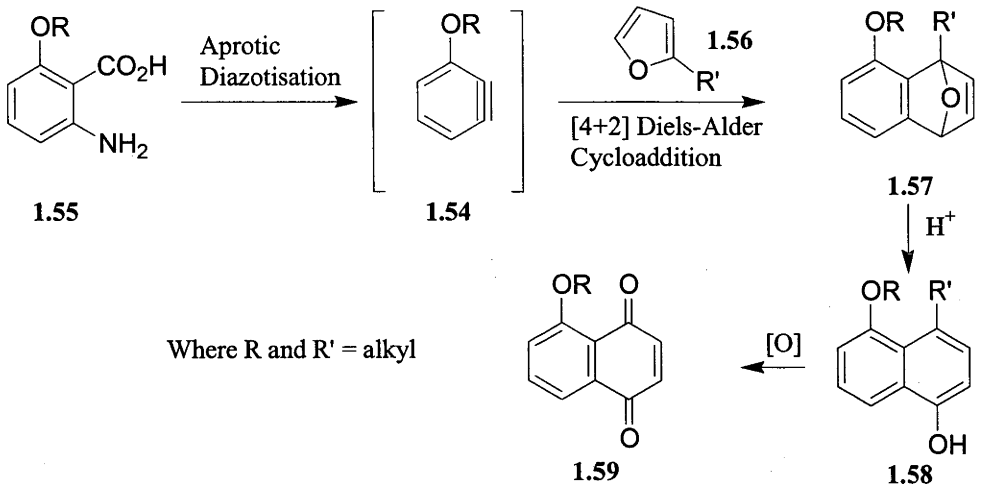
Appropriately functionalised benzoquinones can also be used to access dienes that can participate in Diels-Alder reactions to give naphthoquinones. For example, the Diels-Alder diene **1.51** can be formed via the reaction of a benzoquinone **1.47** with the appropriately substituted alkene **1.52**. The adduct **1.51** thus formed can undergo a Diels-Alder cycloaddition with a second mole of the alkene **1.52**, which participates as the dienophile, to give the desired naphthoquinone **1.53** following aromatisation (Scheme 1.9).



Scheme 1.9 Diels-Alder cycloaddition of alkenes with benzoquinone derivatives

An alternative, general Diels-Alder methodology for the synthesis of naphthoquinones has involved the use of furans as dienes with a benzyne intermediate as the dienophile. An example of this approach is outlined in Scheme 1.10. The benzyne **1.54** is initially generated *in situ* via aprotic diazotisation of the appropriate aniline precursor **1.55**

followed by the decarboxylative elimination of nitrogen. The benzyne **1.54** can then participate in a Diels-Alder cycloaddition with an appropriately substituted furan **1.56**. The major adduct **1.57** thus formed can then undergo acid catalysed ring-opening reactions to give the corresponding naphthol derivative **1.58**, which can be oxidized to give naphthoquinone **1.59**. This methodology has been applied to the synthesis of a number of naphthols and naphthoquinones and various substitution patterns on the ring systems have been achieved.



Scheme 1.10 Diels-Alder cycloaddition of benzynes with furans

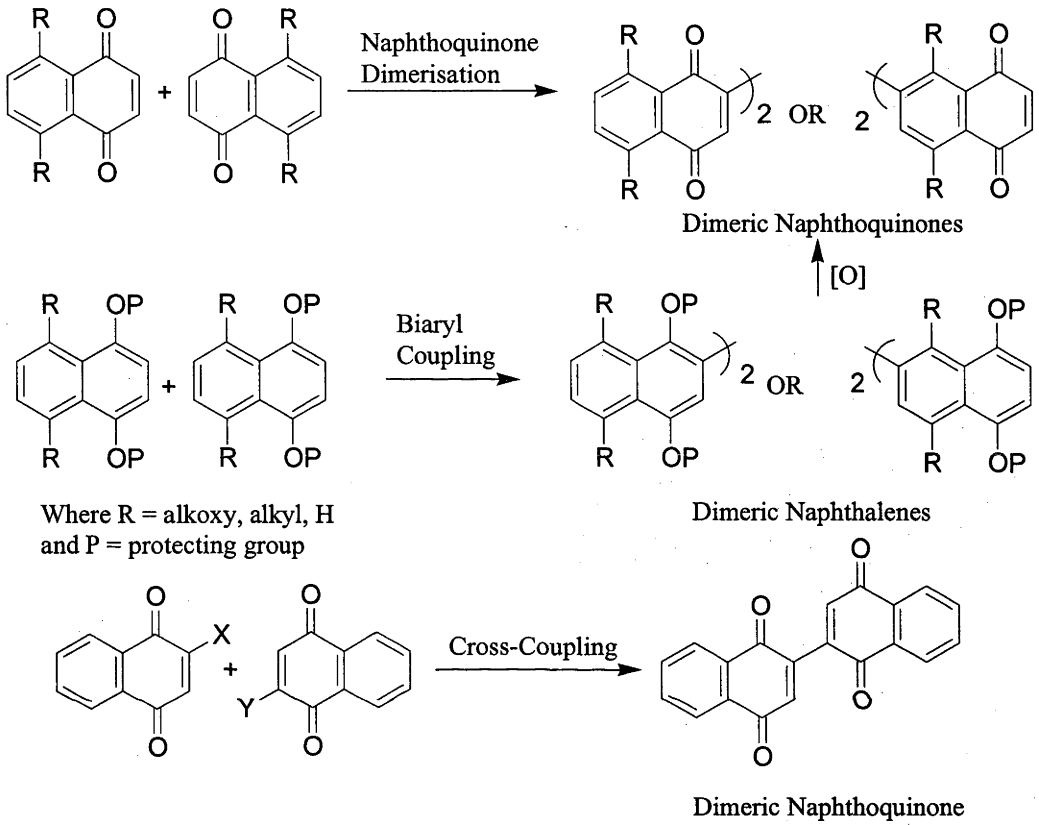
These examples illustrate the versatility of the Diels-Alder approach to the construction of the bicyclic framework via the concerted formation of two new carbon-carbon bonds.

1.3.3 The Synthesis of Dimeric Naphthoquinones

In contrast to the methods for the synthesis of the bicyclic core of naphthoquinones, synthetic routes to dimeric naphthoquinones are more limited. Generally, three approaches have been utilised: the dimerisation of naphthoquinone precursors; the dimerisation of aryl precursors followed by oxidation; and the cross-coupling of tailored naphthoquinone fragments (Scheme 1.11).

The dimerisation of naphthoquinone precursors utilising a number of reagents and conditions has been reported. However, the successful examples usually involve simple naphthoquinones such that the functional group tolerance for these dimerisations has not been explored extensively.

Given the wealth of information available regarding biaryl coupling, many synthetic approaches have involved the construction of the dimeric linkage to afford a biaryl system, prior to oxidation to give the corresponding dimeric naphthoquinone.[104] The regiochemistry of the resultant *bis*-naphthoquinones is usually dictated by the substitution pattern of the monomeric precursors utilised.



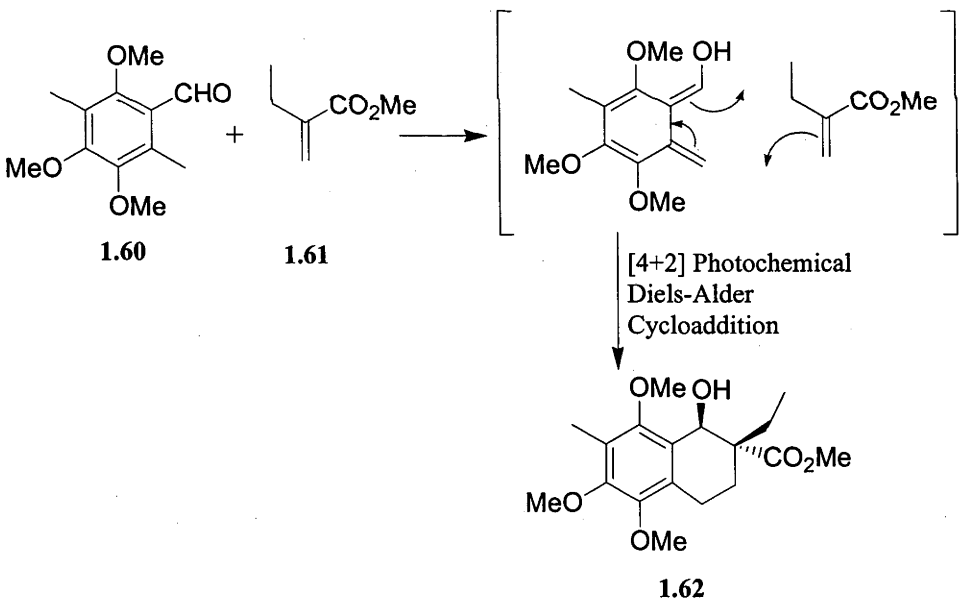
Scheme 1.11 Synthetic routes to bis-*naphthoquinones*

Finally, several recent syntheses of *bis*-naphthoquinones have utilized metal mediated cross-coupling methodology.[105] This approach is inherently regioselective and therefore leads to the directed construction of the dimeric framework. Unfortunately, these protocols require the synthesis of two distinct monomeric precursors and therefore require a greater synthetic effort than the previous alternative routes.

1.4 The Total Synthesis of Hybocarpone (1.25)

In 2001, Nicolaou *et al.* reported the total synthesis of hybocarpone (**1.25**), a dimeric naphthoquinone derivative, incorporating some of the key synthetic approaches discussed above.[106] In general terms, the Nicolaou *et al.* synthesis of hybocarpone (**1.25**) involves two pivotal steps: the construction of the bicyclic framework via a Diels-Alder approach and the dimerisation of the appropriately functionalised naphthoquinone precursor to afford a *bis*-naphthoquinone derivative.[107] This dimerisation strategy is not stereospecific and it is expected that dimerisations of this type would give rise to a number of diastereomeric products.

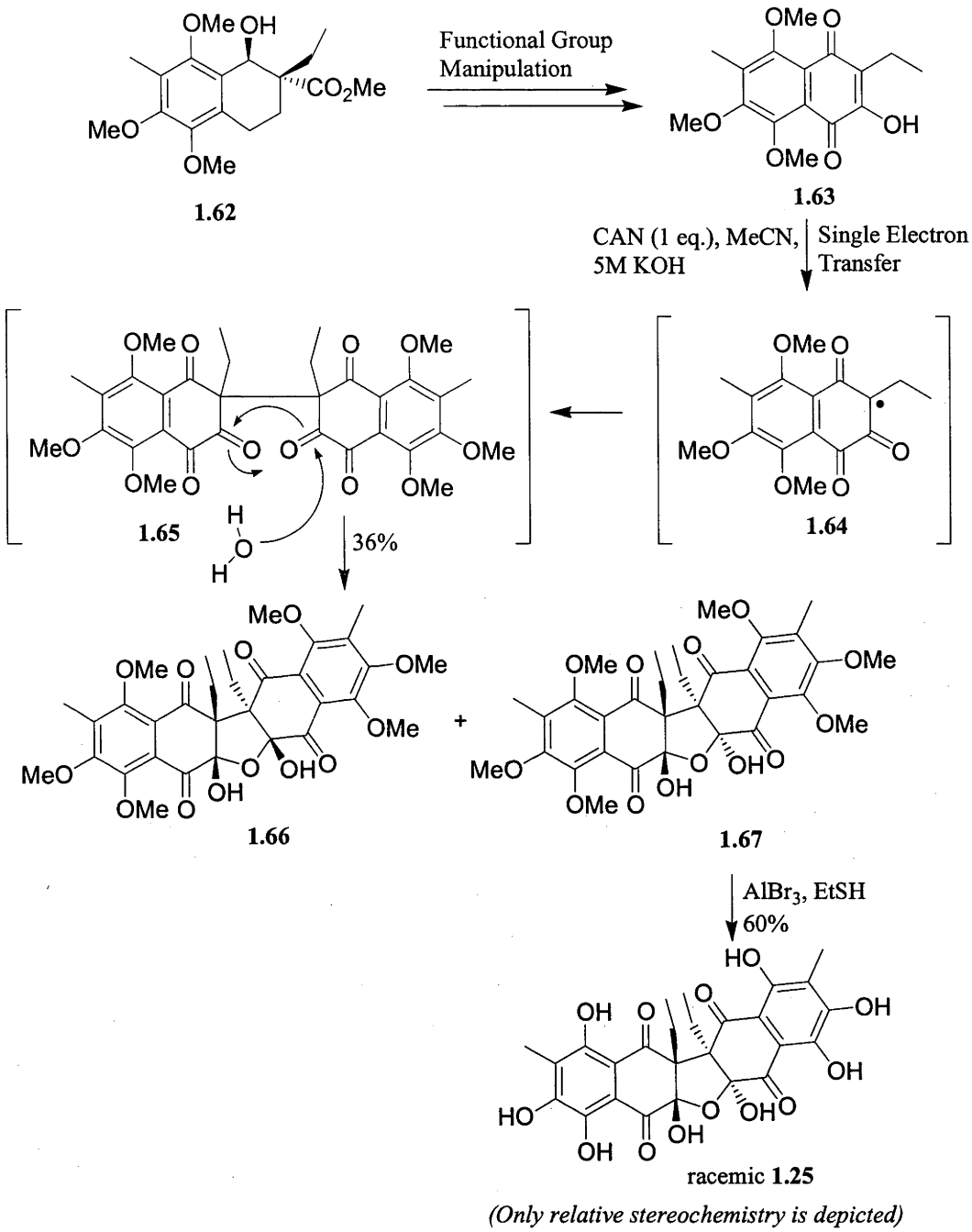
More specifically, the key photochemical Diels-Alder reaction involved the use of the 2-methylbenzaldehyde **1.60**[108] as a diene which underwent the cycloaddition reaction with the appropriately substituted dienophile **1.61**, to give the synthetic intermediate alcohol **1.62**. This novel cycloaddition reaction is thought to proceed through an *ortho*-quinone methide as depicted in Scheme 1.12. In this manner, the construction of the bicyclic structural core was achieved.



Scheme 1.12 Photochemical Diels-Alder reaction mechanism

The secondary alcohol **1.62** was then converted into hydroxy naphthoquinone **1.63** over several synthetic steps involving various functional group manipulations. The treatment of hydroxy naphthoquinone **1.63** with ceric ammonium nitrate then afforded a dimeric naphthoquinone derivative. The proposed mechanism is outlined in Scheme 1.13.

Oxidant induced SET resulted in the generation of radical species **1.64**, and carbon-carbon radical coupling then gave rise to the dimeric derivative **1.65**. Water mediated hemiacetal formation proceeded to give rise to the pentacyclic structural core. The diastereomeric dimers **1.66** and **1.67** were then isolated in poor yield (2 :3 ratio) and could be separated chromatographically. When derivative **1.66** was exposed to slightly acidic conditions, epimerisation gave the thermodynamically stable isomer **1.67**, which possesses analogous relative stereochemistry to the natural product. Treatment of the dimer **1.67** with aluminium tribromide resulted in methyl ether cleavage to afford a compound that was spectroscopically identical to the naturally occurring hybocarpone (**1.25**). It should be noted that lichen-derived hybocarpone (**1.25**) is present in only one stereoisomeric form and is optically active.



Scheme 1.13 The Nicolaou et al. *synthesis of racemic hybocarpone (**1.25**)*

The Nicolaou *et al.* synthesis of racemic hybocarpone (**1.25**) is an elegant example of the use of both a Diels-Alder cycloaddition reaction and an oxidative dimerisation reaction in natural product synthesis. This synthetic route is not, however, readily amenable for the synthesis of analogues of hybocarpone (**1.25**).

1.5 Aims of the Project

In view of the numerous natural products containing substituted naphthazarin moieties, a general synthetic route to these compounds was highly desirable. In particular, the aim of this project was to develop a versatile synthetic route to the natural products aureoquinone (**1.14**) and 2-ethyl-3,6-dihydroxynaphthazarin (**1.15**), as well as naphthazarin **1.16** and other analogues. Synthetic access to naphthazarin **1.16** could clarify the structure of boryquinone through a comparison of the spectral data.

The synthesis of naturally occurring dimeric naphthazarins was also to be investigated, with a view to synthesising the *bis*-naphthazarin **1.20**, islandoquinone (**1.24**) and hybocarpone (**1.25**). Given the successful synthesis of these natural products, their anti-proliferative activity against human breast cancer cells was then to be examined.

The Nicolaou *et al.* racemic synthesis of hybocarpone (**1.25**) was published during the course of this investigation. Their synthetic approach differed significantly from the synthetic route investigated in the present work. More importantly, the Nicolaou *et al.* synthesis was specific for the synthesis of hybocarpone (**1.25**) and could not readily be adapted to access our other synthetic targets. For these reasons, we continued with our synthetic programme.

Chapter Two describes the total synthesis of the naphthazarins aureoquinone (**1.14**), 2-ethyl-3,6-dihydroxy-7-methylnaphthazarin (**1.15**) and boryquinone (**1.16**). The selective, formal total synthesis of hybocarpone (**1.25**) is described in Chapter Three. The attempted synthesis of the naturally occurring *bis*-naphthazarins derivatives is discussed in Chapter Four together with studies on model compounds, while Chapter Five describes the biological testing of the synthetically derived natural products.

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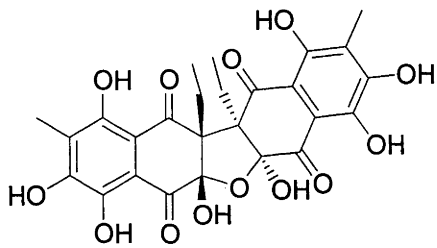
Chapter Two:
The Synthesis of Naturally Occurring Naphthazarins and the Formal Total Synthesis of Hybocarpone

2.1 Introduction

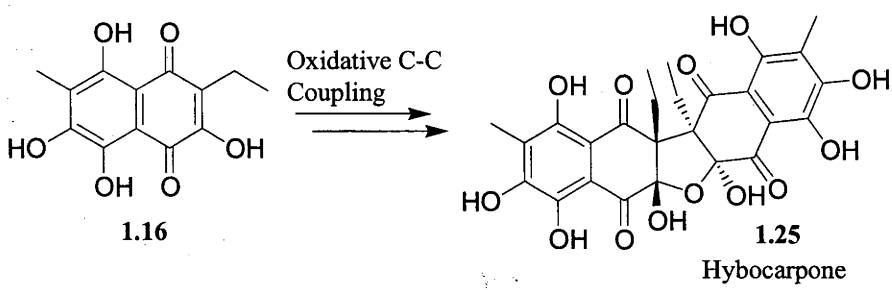
Hybocarpone (**1.25**) is a *bis*-naphthazarin derivative that was isolated by Elix *et al.* from the mycobiont cultures of *Leconara hybocarpa* (Tuck.) Brodo at the Australian National University in 1999 (Figure 2.1).[1] Due to interesting preliminary biological results, further investigation of the bioactivity of hybocarpone (**1.25**) is warranted. A synthetic route to hybocarpone (**1.25**), therefore, was considered an attractive means to access the natural product, due to the paucity from natural sources.



Only relative stereochemistry is known.

*Figure 2.1 Hybocarpone (**1.25**)*

The biosynthesis of hybocarpone (**1.25**) may involve a monomeric precursor such as 3-ethyl-2,7-dihydroxy-6-methyl-1,4-naphthazarin (**1.16**). The oxidative coupling of this putative monomer could result in the formation of a dimeric naphthazarin derivative. Subsequent water-mediated ring closure could conceivably install the central furanoid ring system and result in the formation of hybocarpone (**1.25**) (Scheme 2.1).

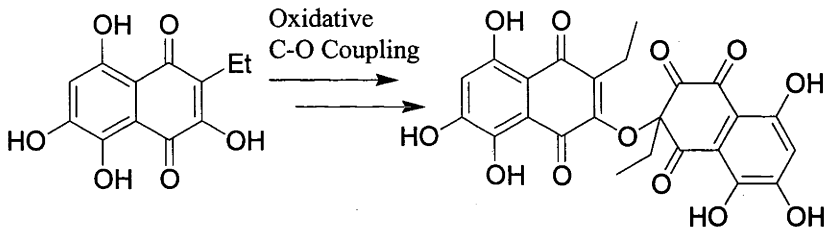


*Scheme 2.1 Postulated biosynthetic route to hybocarpone (**1.25**)*

Huneck *et al.* have reported the isolation of boryquinone from *Cladonia boryi* (Tuck.) Cladoniaceae.[2] The structure of boryquinone was not definitively assigned but the proton NMR spectrum reported was indicative of a dihydroxynaphthazarin containing an ethyl and a methyl substituent. This evidence suggested to us that the structure of boryquinone could be related to that of the postulated biosynthetic precursor to

hybocarpone, naphthazarin **1.16** (Scheme 2.1). We wished to test this hypothesis through the spectroscopic comparison of synthetically derived naphthazarin **1.16** with the natural product boryquinone, which was kindly provided by the authors.

Stepanenko *et al.* have recently reported the isolation of the *bis*-naphthazarin derivative islandoquinone (**1.24**) from the lichen *Cetraria islandia* var. *polaris*.[3] The biosynthesis of islandoquinone (**1.24**) presumably involves the oxidative dimerisation of 3-ethyl-2,7-dihydroxy-1,4-naphthazarin (**1.15**), which was isolated by Moore *et al.* during degradation studies involving the echinoid pigment spinochrome A (Scheme 2.2).



1.15 **1.24** Islandoquinone

*Scheme 2.2 Postulated biosynthetic route to islandoquinone (**1.24**)*

A structurally related fungal metabolite aureoquinone (**1.14**) has recently been isolated from surface cultures of an *Aureobasidium* sp. grown on a synthetic medium.[4] Aureoquinone (**1.14**) displays moderate anti-microbial activity and inhibits a number of different proteases.

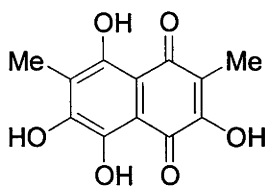


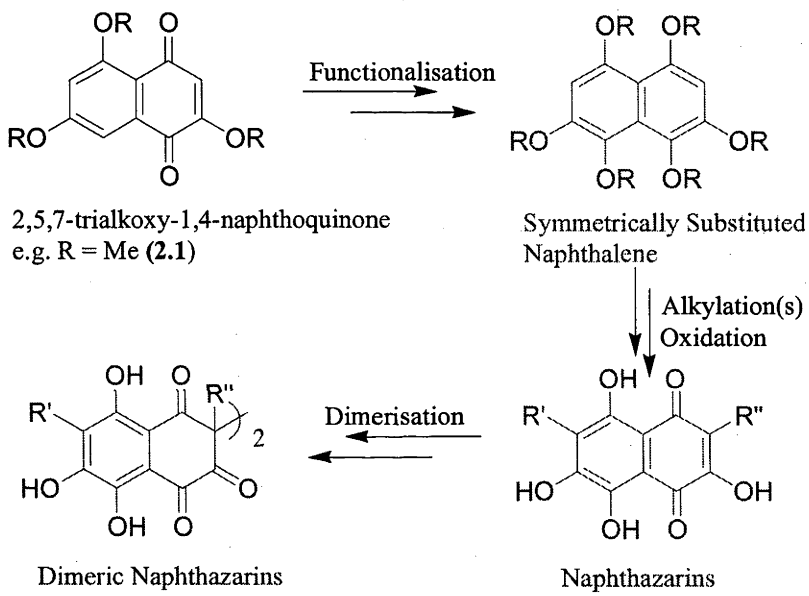
*Figure 2.2 Aureoquinone (**1.14**)*

2.2 Synthetic Strategy Towards the Synthesis of Naturally Occurring Naphthazarins and Bis-*Naphthazarin Derivatives*

Synthetic access to these natural products was considered attractive, as a number of naturally occurring naphthazarins have been isolated to date and this class of compounds generally exhibit bioactivity. In particular, the development of a divergent synthetic pathway that will allow access to various naphthazarins and that could be

extended to incorporate the synthesis of *bis*-naphthazarin derivatives, such as hybocarpone **(1.25)** and islandoquinone **(1.24)**, was desired.

The relatively simple 2,5,7-trialkoxy-1,4-naphthoquinone was identified as a key synthetic precursor as compounds of this type are accessible and generally provide good platforms for further structural manipulation. The regioselective functionalisation of the 2,5,7-trialkoxy-1,4-naphthoquinone should then allow for the synthesis of symmetrically substituted naphthalenes, the appropriate alkylation and oxidative deprotection of which could give rise to naphthazarins, including several natural products. The oxidative dimerisation of the appropriate naphthazarins may subsequently lead to the synthesis of *bis*-naphthazarin derivatives (Scheme 2.3).

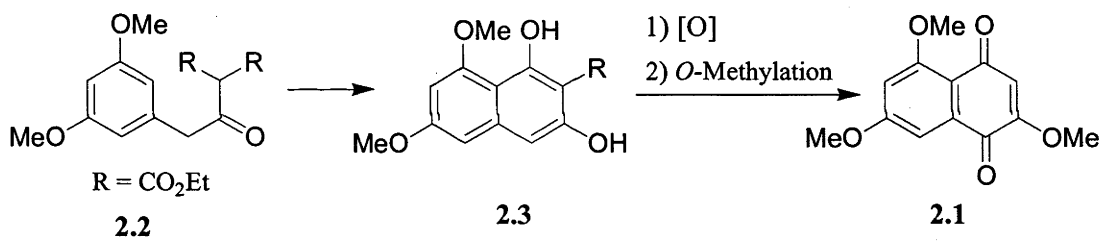


Scheme 2.3 Synthetic approach to naphthazarins and bis-*naphthazarin derivatives*

The simplest appropriate trialkoxy-1,4-naphthoquinone reported to date is 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**). There are several ways in which the naphthoquinone framework has been assembled to afford quinone **2.1**, the *O*-methyl ether of flaviolin, a natural product that has generated much synthetic and medicinal interest.[5] Sections 2.3 and 2.4 will discuss our efforts towards the synthesis of naphthoquinone **2.1**, in light of the relevant reports.

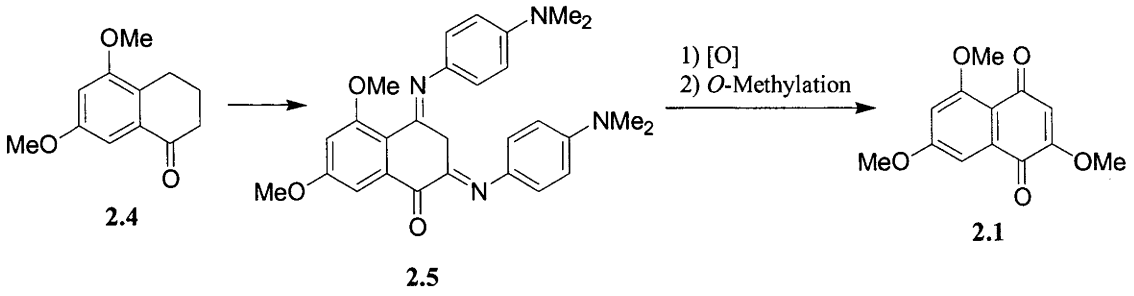
During their seminal work on the 'Acetate Hypothesis', Birch *et al.* examined the oxygenation pattern present in several naturally occurring quinones and attempted to comprehensively relate this to the biosynthetic origins of the compounds. They predicted the regiochemistry of flaviolin accordingly and supported their hypothesis by comparing synthetically derived naphthoquinone **2.1** with material derived from the natural source.[6]

The Birch *et al.* synthesis of naphthoquinone **2.1** commenced from the diester **2.2**, the intramolecular cyclisation of which gave the naphthol **2.3**, which was subsequently oxidised to construct the 1,4-naphthoquinone framework (Scheme 2.4). The *O*-methylation of the naphthoquinone thus formed resulted in the synthesis of the desired naphthoquinone **2.1**. While this synthesis is relatively expedient, the low overall yield achieved by the authors led us to seek an alternative approach to the synthesis of naphthoquinone **2.1**.[6]



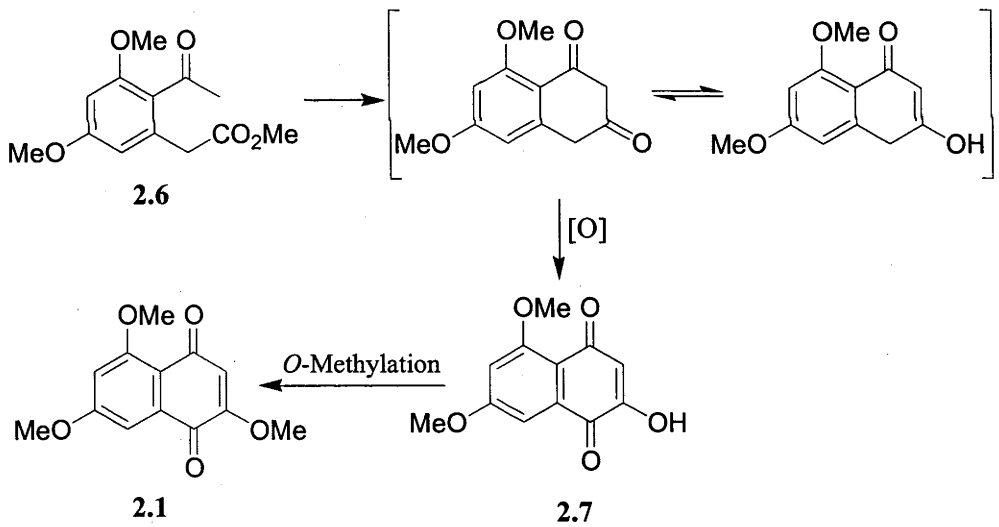
Scheme 2.4 Birch et al. *approach to the synthesis of naphthoquinone* **2.1**

Davies *et al.* contemporaneously reported a synthesis of naphthoquinone **2.1** that involved the construction of the naphthoquinone framework through the appropriately substituted aromatic ketone **2.4** (Scheme 2.5).[7] The aromatic ketone **2.4** was converted to the corresponding dianil **2.5**, the oxidation and *O*-methylation of this adduct then gave the desired naphthoquinone **2.1**. This synthetic route is significantly more laborious than that of Birch *et al.* and, unfortunately, the overall yield reported was just 0.13%. Given that we require significant quantities of naphthoquinone **2.1** for our synthetic studies, this route was unacceptable for our purposes.



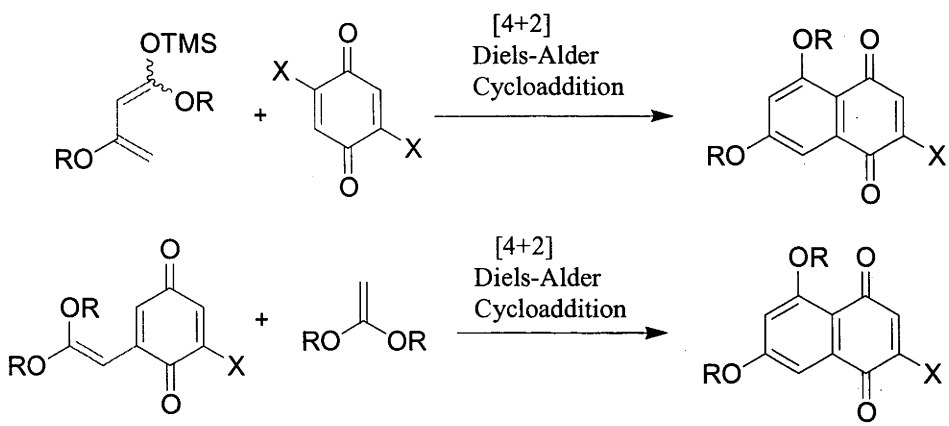
Scheme 2.5 Davies et al. *approach to the synthesis of naphthoquinone* ***2.1***

Bycroft *et al.* reported an alternative synthesis of naphthoquinone **2.1** during their investigations into naturally occurring naphthoquinones.[8-10] The deprotonation of an acyclic aromatic ketone **2.6** at the α-carbon and subsequent intramolecular Claisen cyclisation resulted in the formation of a bicyclic adduct, the *in situ* aerial oxidation of which gave the 2-hydroxy-1,4-naphthoquinone **2.7**. The *O*-methylation of naphthoquinone **2.7** then allowed for the synthesis of the desired product, naphthoquinone **2.1** (Scheme 2.6).



Scheme 2.6 Bycroft et al. *approach to the synthesis of naphthoquinone* ***2.1***

Brassard *et al.* have developed a number of approaches to the desired naphthoquinone **2.1**, and related compounds, based on cycloaddition reactions of functionalised benzoquinones.[11-17] The benzoquinones utilised during their investigations could participate in intermolecular [4+2] Diels-Alder cycloaddition reactions as either the diene or the dienophile, as depicted in Scheme 2.7. These synthetic routes will be discussed in further detail in Sections 2.4.1 and 2.4.2.



Where R = alkyl and X = halo, alkoxy

Scheme 2.7 Naphthoquinone synthesis based on the reactions of benzoquinones

We chose to consider the work of Brassard *et al.* at the outset of our synthetic studies due to their good overall yields, easily accessed starting materials and the scope within their approaches for analogue synthesis.

2.4 The Synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone **2.1**

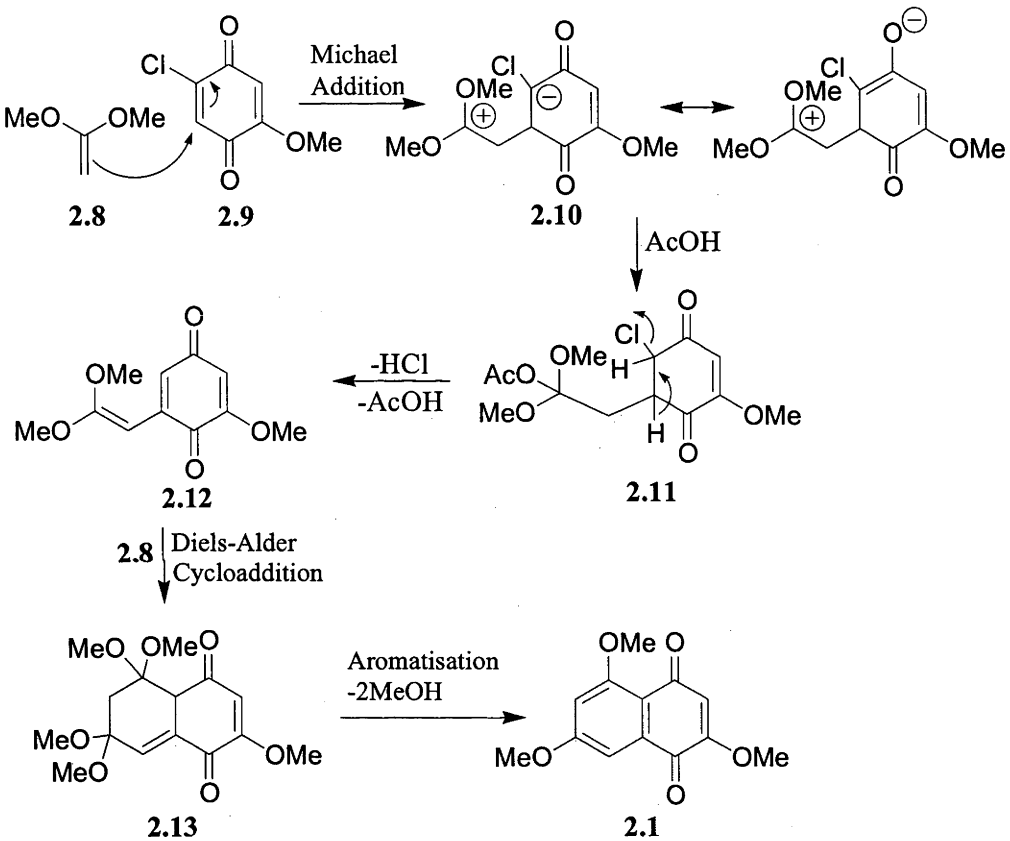
2.4.1 The Synthesis of Naphthoquinone **2.1** *via a Diels-Alder Cycloaddition Reaction*

The 1,4-naphthoquinone structural framework has been accessed synthetically from benzoquinone precursors via Diels-Alder approaches. The most direct of these synthetic strategies initially involves the nucleophilic addition of a 1,1-dialkoxyethene to a benzoquinone, in a Michael addition fashion, to form the Diels-Alder diene under acidic conditions. The concerted [4+2] cycloaddition reaction of this diene with the 1,1-dialkoxyethene *in situ* then gives rise to the corresponding naphthoquinone.

The key synthetic target, 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**), has been synthesised accordingly via the reaction of 1,1-dimethoxyethene (**2.8**) with 2-chloro-5-methoxy-1,4-benzoquinone (**2.9**) under acidic conditions. The mechanism for this synthesis is outlined in Scheme 2.8. The nucleophilic addition of 1,1-dimethoxyethene (**2.8**) to the benzoquinone **2.9** affords the zwitterionic adduct **2.10**. The regioselectivity observed arises from the different electronic properties of the chloro and methoxy substituents on the benzoquinone **2.9**. As the chloro functionality is more electron

withdrawing, the nucleophilic attack of 1,1-dimethoxyethene (**2.8**) is directed at the more electron deficient double bond of the benzoquinone **2.9**.

In the presence of acetic acid, the intermediate adduct **2.11** can then be obtained. The subsequent elimination of hydrochloric acid and acetic acid then leads to the formation of diene **2.12**, which can participate in a [4+2] Diels-Alder cycloaddition reaction with a second equivalent of 1,1-dimethoxyethene (**2.8**). Aromatisation of the resultant bicyclic adduct **2.13** results in the synthesis of the desired naphthoquinone **2.1**.[18]

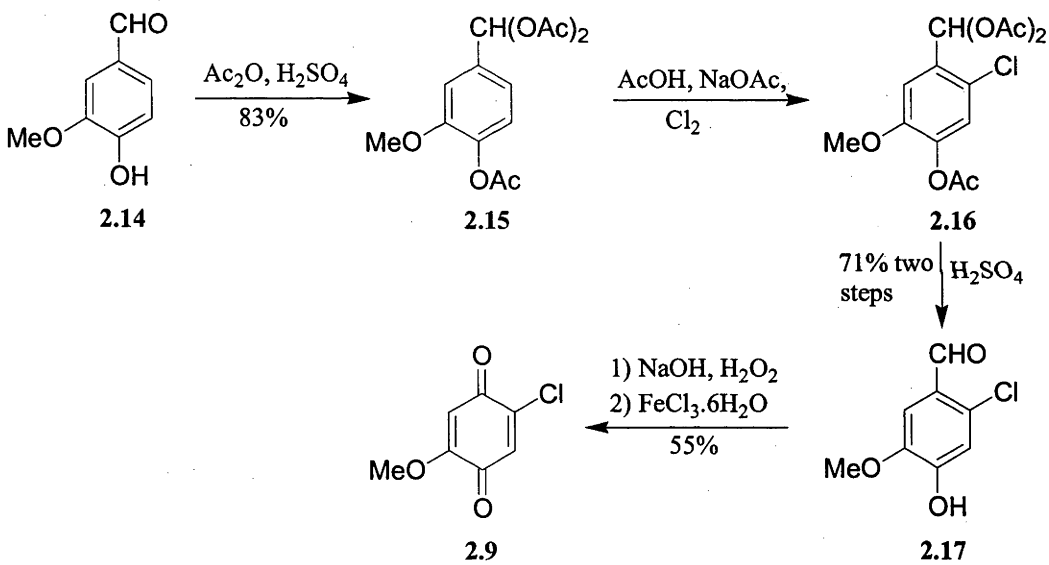


Scheme 2.8 Proposed mechanism for synthesis of naphthoquinone ***2.1***

As this was the most direct reported synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**), this methodology was utilised for our own synthetic purposes. The required benzoquinone **2.9** was therefore synthesised according to previously developed procedures. The aromatic aldehyde *p*-vanillin (**2.14**) was initially masked as the corresponding acetyl acetal **2.15**, under acidic conditions, through treatment with acetic anhydride. This synthetic step was necessary in order to control the regioselectivity of the subsequent halogenation, as direct chlorination of aromatic aldehyde **2.14** reportedly results in the installation of the chloro group *ortho* to the hydroxy functionality, a strong

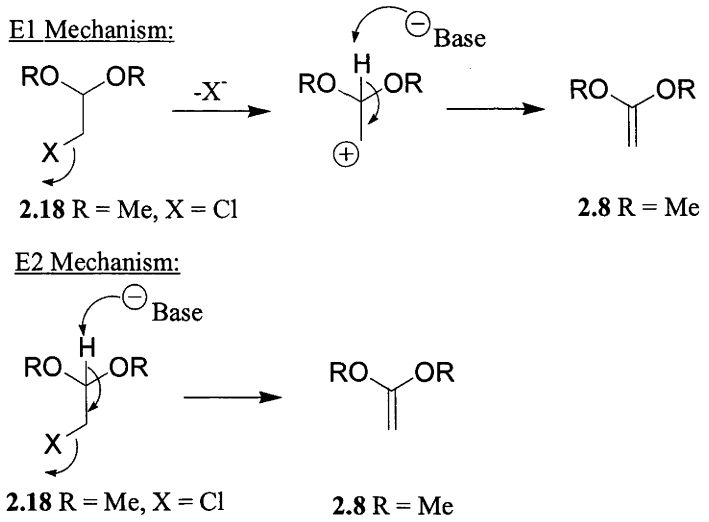
ortho-para directing group.[19] The treatment of the acetyl acetal **2.15** with chlorine gas then gave the appropriate chlorobenzene **2.16**, as regioselective electrophilic aromatic substitution at the position *para* to the methoxy substituent was observed. The chlorinated adduct **2.16** was then efficiently deprotected under acidic conditions to afford the phenol **2.17**, in an overall yield of 71% from the acetyl acetal **2.15** (Scheme 2.9).

Following the methodology developed by Asp and Lindberg, the aldehyde functionality was subsequently oxidised under Dakin oxidation conditions (alkaline hydrogen peroxide) to give the corresponding hydroquinone after hydrolysis.[20] The hydroquinone was immediately oxidised with iron trichloride to give the desired benzoquinone **2.9**. The benzoquinone **2.9** precipitated as pure crystals from an aqueous solution in an overall yield of 32% over five synthetic steps.



Scheme 2.9 The synthesis of benzoquinone 2.9

Following the synthesis of benzoquinone **2.9**, access to 1,1-dimethoxyethene (**2.8**) was sought. Dialkoxyethenes have generally been synthesised via the treatment of a haloacetaldehyde dialkylacetal derivative, such as acetal **2.18**, with a strong base. This reaction can proceed through either an E1 or an E2 mechanism as depicted in Scheme 2.10.



Scheme 2.10 The synthesis of dialkoxyethenes via haloacetaldehyde dialkylacetals

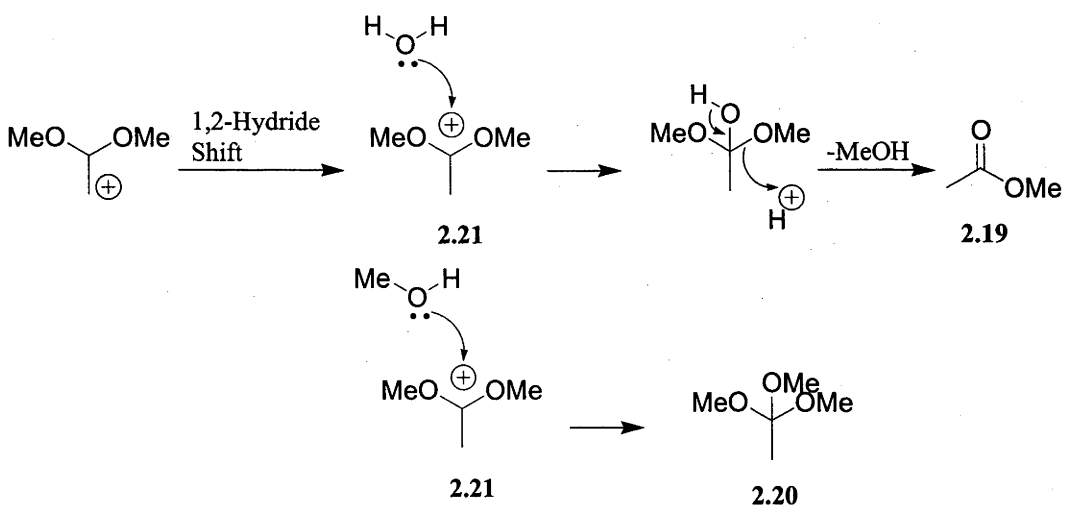
As a variety of bases and conditions have been utilised for the synthesis of dialkoxyethenes, our work focused on assessing suitable conditions for this transformation. These results are summarised in Table 2.1. Initial attempts to synthesise 1,1-dimethoxyethene (**2.8**) from the commercially available chloroacetaldehyde dimethylacetal (**2.18**) utilised potassium *tert*-butoxide in *tert*-butyl alcohol under the conditions developed by McElvain for an analogous conversion (Entry 1, Table 2.1).[21] This reaction was, unfortunately, unsuccessful in our hands, as the formation of dialkoxyethene **2.8** was not evident. The discrepancy between our experimental results and those of McElvain cannot be readily rationalised, although the dialkoxyethene **2.8** is known to polymerise when heated.[22]

When the reaction was repeated at -78°C in THF (Entry 2, Table 2.1) starting material was recovered quantitatively. Likewise, the desired alkene **2.8** was not evident when chloroacetaldehyde dimethylacetal (**2.18**) was treated with potassium *tert*-butoxide at 0°C in the presence of 18-crown-6 (Entry 3, Table 2.1).

Table 2.1 Conditions employed Towards the Synthesis of 1,1-dimethoxyethene (2.8)

Entry	Base/Additive	Solvent	Temperature/Conditions	Observations
1	*t*-BuOK	*t*-BuOH	Reflux	No reaction
2	*t*-BuOK	Et_2O	-78°C to R.T.	No reaction
3	*t*-BuOK/ 18-crown-6	THF	0°C	No reaction
4	Na pinacolate	Pinacol	100°C	**2.19** and **2.20**
5	K α-terpineate	α-terpineol	40°C	**2.8** (86%)
6	KOH/TBAB	DMSO	60°C/sonication	No reaction
7	KOH/TBAB	DMSO	130-140°C	**2.8** (74%)

McElvain *et al.* have also reported the use of sodium pinacolate for the synthesis of similar dialkoxyethenes.[23] When we treated chloroacetaldehyde dimethylacetal (**2.18**) with sodium pinacolate, however, the formation of methyl acetate (**2.19**) and 1,1,1-trimethoxyethane (**2.20**) was evident (Entry 4, Table 2.1). It is likely that the by-products are formed from the trapping of the intermediate carbocation **2.21** with water to give methyl acetate (**2.19**), after the elimination of methanol, or with the methanol formed *in situ* to give 1,1,1-trimethoxyethane (**2.20**) (Scheme 2.11).



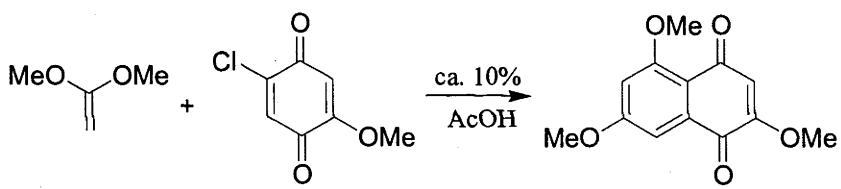
Scheme 2.11 Proposed mechanism for the formation of observed reaction by-products

When potassium terpineate was used instead of sodium pinacolate as the base, an 86% conversion to the desired alkene **2.8**, was observed (Entry 5, Table 2.1). Purification of

the 1,1-dimethoxyethene (**2.8**) by fractional distillation was somewhat difficult, however, due to contamination with α-terpineol.

An analogous reaction involving the use of potassium hydroxide and tetrabutylammonium bromide (TBAB) in dimethyl sulfoxide (DMSO) with sonication has recently been reported.[24] When we employed these conditions only starting material was recovered (Entry 6, Table 2.1), the application of heat, however, resulted in the synthesis of the desired product in 74% yield. The 1,1-dimethoxyethene (**2.8**) was subsequently purified by distillation under atmospheric pressure at 88-89°C.

Having obtained both 1,1-dimethoxyethene (**2.8**) and benzoquinone **2.9**, the synthesis of naphthoquinone **2.1** was attempted according to the procedure developed by Brassard *et al.* (Scheme 2.12).[18] Analysis of the proton NMR spectrum of the reaction mixture indicated that the desired product was formed, due the presence of a characteristic naphthoquinonoid proton singlet at 6.00 ppm. The poor yield obtained, and difficulty experienced in the purification of the product, led us abandon this approach.

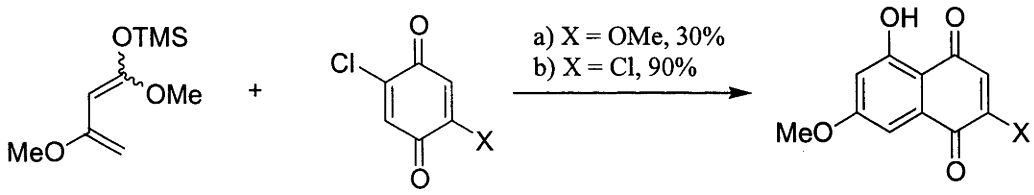


2.8 **2.9** **2.1**

*Scheme 2.12 The synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone **2.1***

*2.4.2 An Alternative Diels-Alder Approach to the Synthesis of Naphthoquinone **2.1***

The 1,4-naphthoquinone framework has also been assembled via the Diels-Alder cycloaddition reaction of diene **2.22** with either 2-chloro-5-methoxy-1,4-benzoquinone (**2.9**) or 2,5-dichloro-1,4-benzoquinone (**2.23**) (Scheme 2.13).[25]



2.22

2.9 X = OMe
2.23 X = Cl

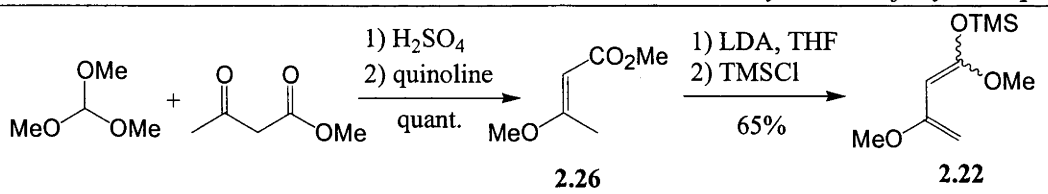
2.24 X = OMe
2.25 X = Cl

Scheme 2.13 Alternative approach to the construction of the naphthoquinone framework

Significantly lower yields are observed for the [4+2] cycloaddition reaction of 2-chloro-5-methoxy-1,4-benzoquinone (**2.9**) with diene **2.22**, than the analogous reaction utilising of 2,5-dichloro-1,4-benzoquinone (**2.23**) as the dienophile. Although both chloro and methoxy substituents are *-I* and +R groups, the alkoxy functionality has a dominant contribution to the energies of the interacting frontier molecular orbitals and thereby governs both the regioselectivity and reactivity of the reaction.[26] Thus, although the use of 2-chloro-5-methoxy-1,4-benzoquinone (**2.9**) as a dienophile will clearly enable access to a naphthoquinone with the oxygenation pattern desired, the higher yields obtained with 2,5-dichloro-1,4-benzoquinone (**2.23**) suggest that the latter route may be more attractive.

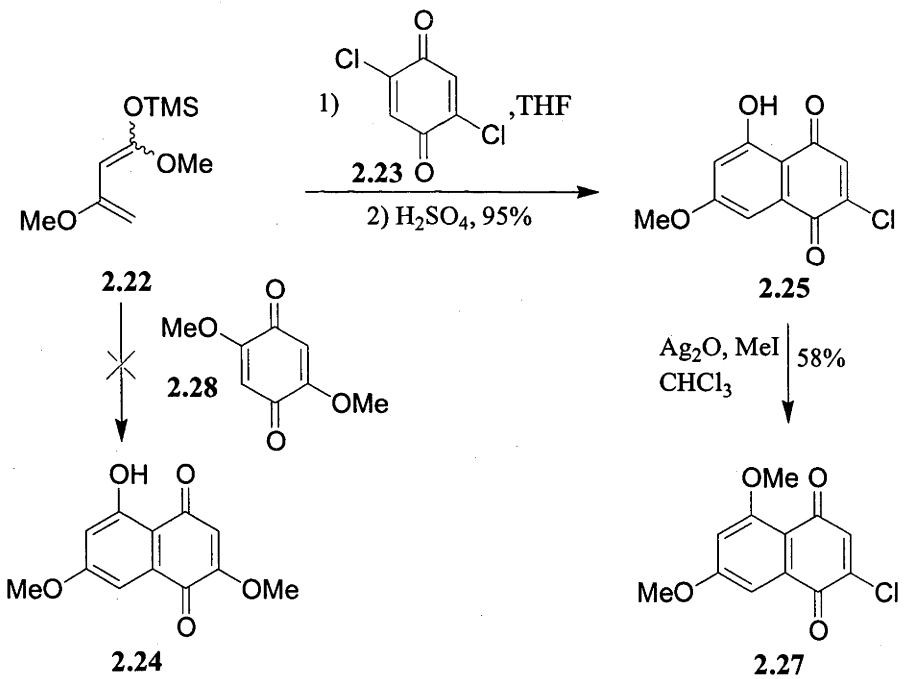
The synthesis of diene **2.22** thereby commenced with the preparation of methyl-(*E*)-methoxy-2-butanoate (**2.26**) through treatment of methyl acetoacetate with trimethylorthoformate under acidic conditions (Scheme 2.14). The acid catalysed enolisation of methyl acetoacetate is thought to precede *in situ* trapping with 'Me^+' from trimethylorthoformate to give the corresponding enol ether **2.26**. Alkene formation was characterised by the appearance of a singlet at δ 5.01 in the proton NMR spectrum.[27]

The alkene **2.26** thus obtained was subsequently deprotonated at the terminal carbon through treatment with lithium diisopropylamine (LDA), and the anion thereby formed was trapped with trimethylsilyl chloride (TMSCl) to give the *O*-TMS protected diene, 1,3-dimethoxy-1-trimethylsiloxybuta-1,3-diene, (**2.22**), in good yield. The diene **2.22** thus prepared was characterised by the appearance of a doublet of doublets at 3.98 ppm in the proton NMR spectrum due to a proton at the C4-position.[17]



Scheme 2.14 The Brassard et al. *synthesis of diene* ***2.22***

The Diels-Alder cycloaddition reaction of diene **2.22** with the benzoquinone **2.23** gave 2-chloro-5-hydroxy-7-methoxy-1,4-naphthoquinone (**2.25**). The isolation of a single regioisomer was consistent with the observations of Brassard and co-workers.[25] The characteristic quinonoid resonance was observed at δ 7.17 for the naphthoquinone **2.25**. The 2-chloro-1,4-naphthoquinone **2.25** was subsequently *O*-methylated through treatment with silver(I) oxide and methyl iodide to give 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) (Scheme 2.15).



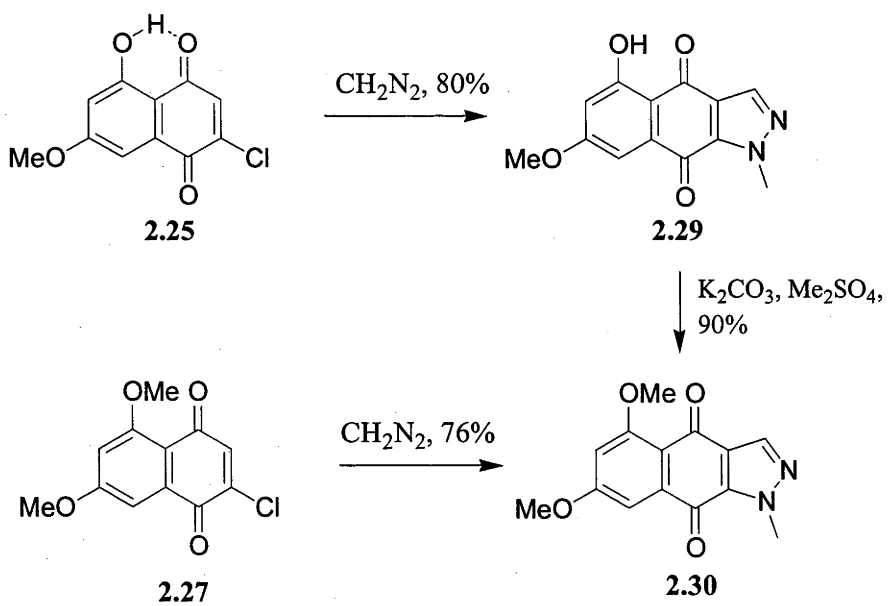
Scheme 2.15 The synthesis of naphthoquinone ***2.27*** *and attempted synthesis of naphthoquinone* ***2.24***

An analogous Diels-Alder cycloaddition reaction between the diene **2.22** and the commercially available 2,5-dimethoxy-1,4-benzoquinone (**2.28**) was also attempted during the course of this investigation. No reaction was observed and benzoquinone **2.28** was isolated from the reaction mixture (Scheme 2.15). This concurs with previous

observations that significantly electron-donating substituents can deactivate the dienophile towards Diels-Alder cycloaddition reactions.

The *O*-methylation of 2-chloro-5-hydroxy-7-methoxy-1,4-naphthoquinone (**2.25**) necessitated the use of large amounts of expensive silver(I) oxide. In an effort to circumvent the use of this reagent, the synthesis of naphthoquinone **2.27** was attempted via treatment of naphthoquinone **2.25** with ethereal diazomethane, as this has been previously employed for similar reactions.[28] Interestingly, we found that *O*-methylation at the phenolic C5-position of naphthoquinone **2.25** did not occur but the naphthoquinone **2.29** was isolated instead. The tricyclic derivative **2.29** is thought to arise from the initial 1,3-dipolar cycloaddition reaction of naphthoquinone **2.25**, with diazomethane followed by *N*-methylation to give the naphthoquinone **2.29** (Scheme 2.16).

When 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) was treated with diazomethane, a similar reaction outcome was observed and the tricyclic adduct **2.30** was obtained. Treatment of the phenol **2.29** with an excess of potassium carbonate and dimethyl sulfate gave the naphthoquinone **2.30**, thus establishing that the regioselectivity of each of the 1,3-dipolar cycloaddition reactions is analogous.

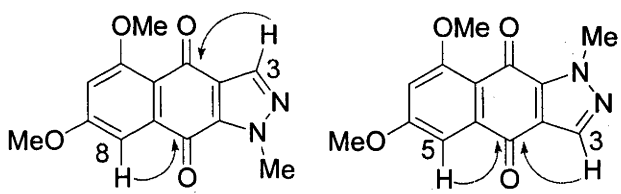


Scheme 2.16 The formation of 1,3-dipolar cycloaddition/N-methylation adducts

These results suggest that the *O*-methylation of the 8-hydroxy-1,4-naphthoquinone **2.25** competes with a [3+2] cycloaddition reaction of the 1,3-dipolar diazomethane with the

quinonoid double bond. This is indicative of a strong hydrogen bond between the phenolic hydrogen and the carbonyl oxygen, due to the formation of a six-membered ring (shown in blue in Scheme 2.16).

In principle, the regiochemical outcome of the 1,3-dipolar cycloaddition reaction could result in the formation of either naphthoquinone **2.30** or **2.31** (Figure 2.3). We initially attempted to establish the regiochemistry of the isolated product using two-dimensional NMR spectroscopy. Figure 2.3 shows the $^{3}J_{CH}$ correlations that would allow for a full regiochemical assignment. For regioisomer **2.30**, it was expected that the proton at the C3-position would display a $^{3}J_{CH}$ correlation with one carbonyl carbon of the quinone ring, while the proton at the C8-position would display a $^{3}J_{CH}$ correlation with the other quinonoid carbonyl carbon. Alternatively, it was expected that the proton at the C3- and C5-positions of the regiosiomer **2.31** would display $^{3}J_{CH}$ correlations with the same carbonyl carbon.



2.30 **2.31**

*Figure 2.3 Possible regioisomeric products **2.30** and **2.31***

As these $^{3}J_{CH}$ correlations were not observed via gHMBC or gHMQC experiments we were unable to assign the regiochemistry of the isolated product. A single crystal was subsequently grown and the structure was determined definitively through X-ray crystallographic analysis. The crystal structure confirmed the regiochemical outcome of the reaction as leading to the synthesis of naphthoquinone **2.30** (Figure 2.4).

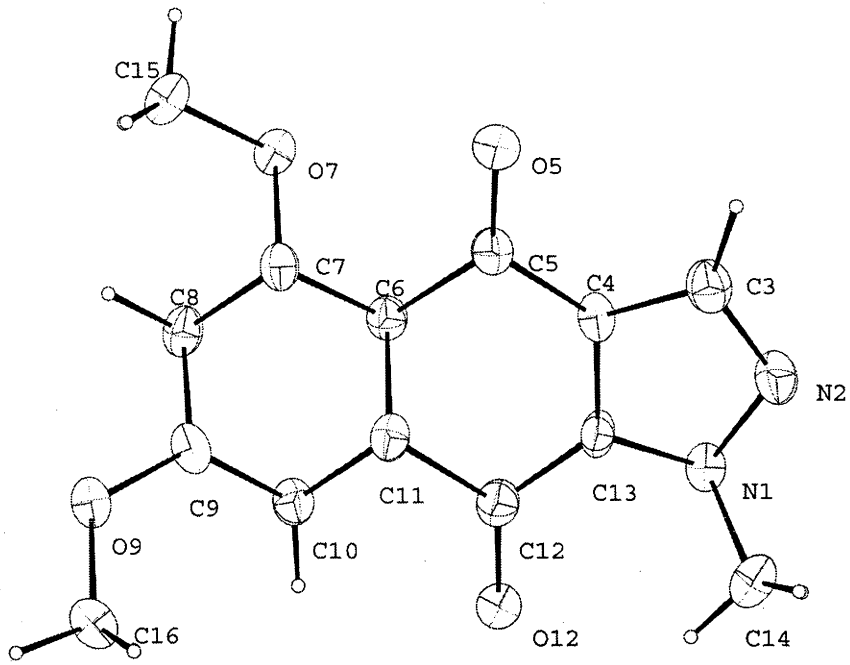
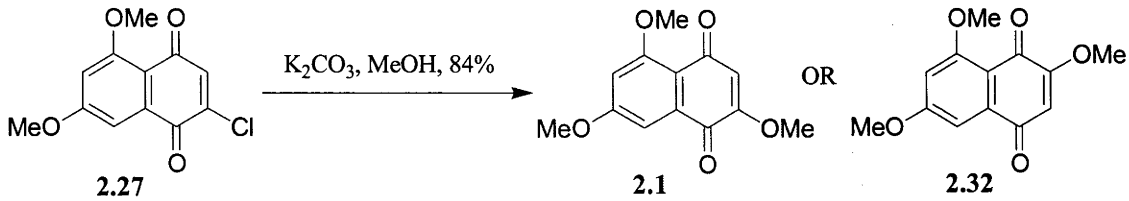


Figure 2.4 Thermal ellipsoid diagram of tricyclic adduct ***2.30*** *with selected atom labelling. Ellipsoids show 50% probability levels. Hydrogen atoms are drawn as circles with small radii. (X-ray analysis performed by Dr Alison Edwards)*

Whilst the use of diazomethane is clearly not suitable for the *O*-methylation of 2-chloro-1,4-naphthoquinones, the naphthoquinone core can be further elaborated in this manner. 1,3-Dipolar cycloaddition reactions of diazomethane with naphthoquinones have, in fact, been observed previously.[29,30] Interestingly, Laatsch *et al.* have utilised the indazolequinones thus formed in the synthesis of alkyl bridged naphthoquinone dimers and so we have, in principle, a means to access dimeric naphthoquinones.[31]

The conversion of 2-chloro-5,7-dimethoxy-1,4-napthoquinone (**2.27**) to 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**) was achieved by Brassard *et al.* through displacement of the 2-chloro substituent on the naphthoquinone moiety using sodium methoxide in benzene. Due to the toxicity of benzene, and the scale on which these reactions are carried out, we attempted this reaction using sodium methoxide in methanol, however, a complex mixture of products ensued. When anhydrous potassium carbonate in anhydrous methanol replaced sodium methoxide, a single regioisomer, naphthoquinone **2.1** or **2.32**, was isolated (Scheme 2.17).



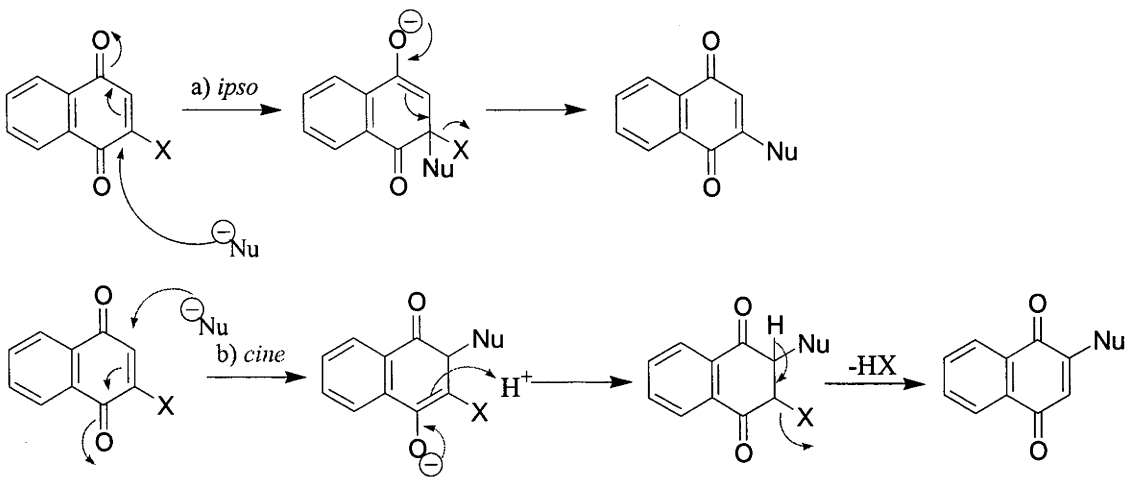
Scheme 2.17 Nucleophilic displacement of the C2-chloro substituent

2.4.3 The Regioselectivity of Nucleophilic Displacement Reactions

Although the spectral data obtained for the naphthoquinone thus isolated were consistent with the data reported by Brassard *et al.*, the regiochemistry of the reported product was assigned based on the availability of both regioisomeric quinones **2.1** and **2.32**. Given the similarity in the spectroscopic data for the two regioisomers, we were unable to unambiguously assign the structure of the product obtained under our reaction conditions.

Our attempts to use two-dimensional NMR spectroscopy techniques (gHMBC and gHMQC experiments) were equally ambiguous, as the two carbonyl groups of the naphthoquinonoid moiety could not be readily distinguished, and thus used in the assignment of regiochemistry. We were also unable to grow a suitable crystal of naphthoquinone **2.1** for X-ray crystallographic analysis.

In principle, the displacement of the quinonoid C2-chloro substituent by the 'methoxide anion' can occur at either the *ipso* position or the *cine* position of a given naphthoquinone (Scheme 2.18). Studies by Cameron *et al.* on simple naphthoquinones have established that nucleophilic attack on 2-chloro-1,4-naphthoquinones by the methoxide anion in methanol gave the *ipso* product in 93% yield.[32]



Scheme 2.18 Regiochemistry and proposed mechanisms of nucleophilic displacement

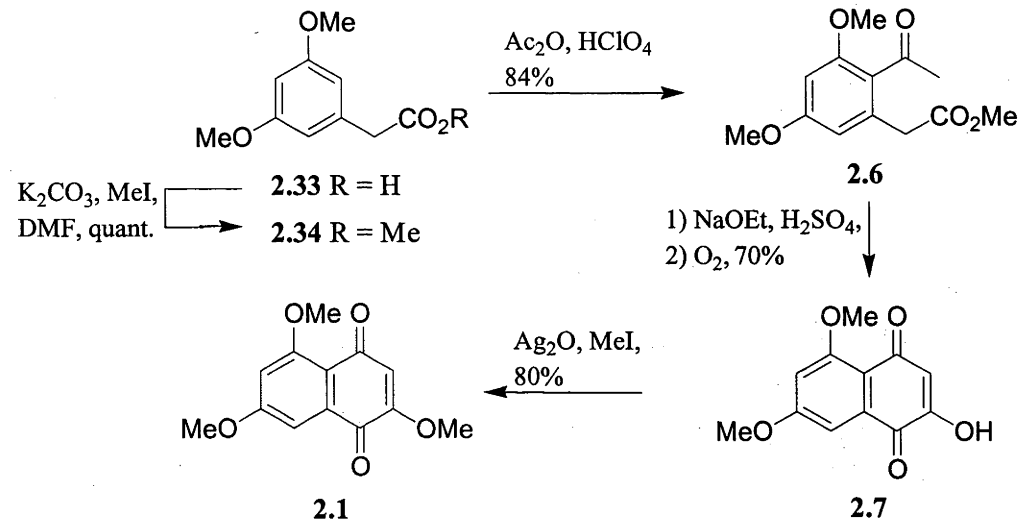
The mechanism depicted in Scheme 2.18 indicates that *cine* attack is facilitated by acidic conditions. The synthesis of the trimethoxy naphthoquinone isolated was carried out under basic conditions, but, to our knowledge, similar studies involving 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) have not been reported and thus the regiochemistry of the adduct obtained cannot be assigned with certainty.

2.4.4 The Synthesis of Naphthoquinone **2.1** via *Claisen Condensation*

In view of this, we embarked upon an alternative, unambiguous synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**) reported by Bycroft *et al.* (Scheme 2.19).[8,10] The synthesis commenced with commercially available 3,5-dimethoxyphenylacetic acid (**2.33**), which was converted quantitatively to the corresponding methyl ester **2.34** through treatment with potassium carbonate and methyl iodide. Formation of the methyl ester **2.34** was evident through the appearance of a methoxy signal at 3.67 ppm in the proton NMR spectrum.

The methyl ester **2.34** was subsequently subjected to a Friedel-Crafts acylation with acetic anhydride using perchloric acid as the catalyst. This acylation occurred regioselectively at the C2-position to give the aromatic ketone **2.6** in 84% yield, due to the complementary directing effect of two methoxy substituents on the aromatic ring. Only monoacylation was observed, presumably due to the deactivating effect of the newly installed, electron withdrawing acyl functional group.

The aromatic ketone **2.6** thus formed was then treated with an excess of sodium ethoxide to effect an intramolecular Claisen condensation. Benzylic oxidation was readily achieved through aerial oxidation and 2-hydroxy-5,7-dimethoxy-1,4-naphthoquinone (**2.7**) was obtained in good yield. The appearance of a quinonoid singlet at 6.02 ppm in the proton NMR spectrum was characteristic of naphthoquinone **2.7**. Subsequent *O*-methylation with silver(I) oxide and methyl iodide gave the desired 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**) in excellent yield.



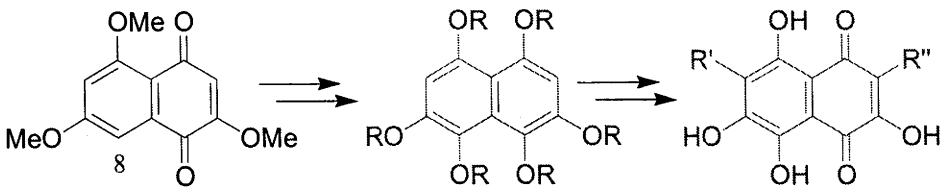
Scheme 2.19 Bycroft et al. *synthesis of key intermediate naphthoquinone* ***2.1***

A comparison of the spectroscopic and chromatographic properties of the naphthoquinone synthesised following the procedures of Brassard *et al.* and Bycroft *et al.* showed that the products obtained were identical. This result confirms the regiochemistry of the nucleophilic displacement reaction discussed in Section 2.4.3. Attack of the 'methoxide anion' generated *in situ* therefore occurred at the *ipso* position to give the desired naphthoquinone **2.1** (Schemes 2.17 and 2.18).

We have therefore successfully synthesised the target naphthoquinone **2.1**, following three procedures that have been previously reported in the literature. The structure of the key intermediate naphthoquinone **2.1** has been established and sufficient quantities of the quinone were accessed through this investigation to enable us to proceed to the next synthetic target, the symmetrically substituted naphthalene (Scheme 2.3).

2.5 The Synthesis of Symmetrically Substituted Naphthalene Intermediate

Following the successful synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**), we sought to further functionalise the naphthoquinone framework so as to access the naturally occurring naphthazarins. A symmetrically substituted naphthalene was then identified as a key target, as it represents the simplest possible system that contains all of the appropriate oxygen functionality appended to the bicyclic core (Scheme 2.20).

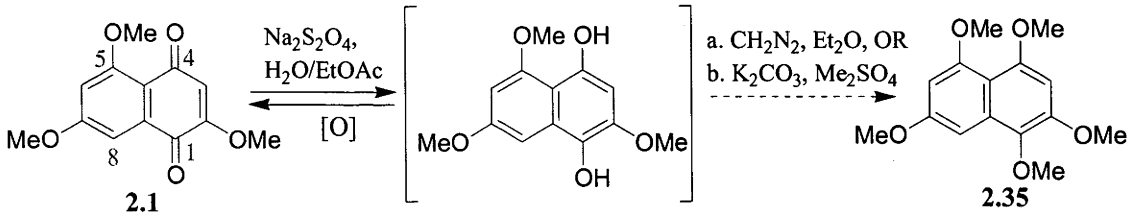


Naphthoquinone **2.1** | Symmetrically Substituted Naphthalenes | Naphthazarins

Scheme 2.20 Synthetic approach to naphthazarins

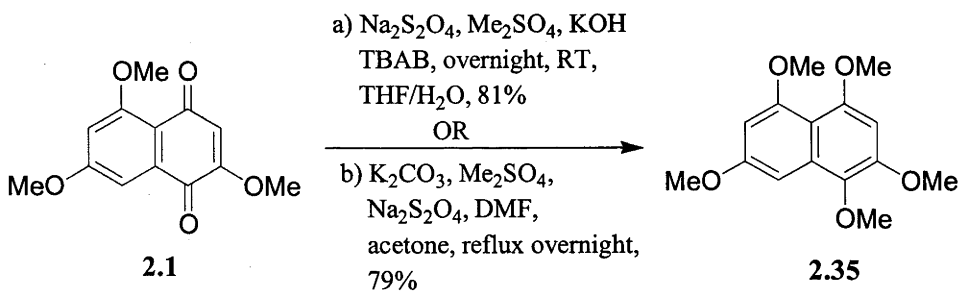
The synthesis of symmetrically substituted naphthalenes from naphthoquinone **2.1** would require the installation of functionality at the C8-position on the bicyclic framework. It was envisaged that this would be most readily achieved via a fully aromatic system and so attempts were made to convert the naphthoquinone **2.1** to the corresponding naphthalene system.

Initially, the reduction of naphthoquinone **2.1**, followed by *O*-protection, was attempted as a two-step process as shown in Scheme 2.21. Naphthoquinone **2.1** was treated with a large excess of aqueous sodium dithionite and the disappearance of the coloured starting material was monitored visually. The resulting colourless reaction mixture was immediately treated with either ethereal diazomethane or dimethyl sulfate/potassium carbonate in an attempt to convert the phenolic hydroxyl groups to the corresponding methyl ethers. Disappointingly, the only compound isolated from these attempts was the starting material, naphthoquinone **2.1**, presumably due to the facile aerial oxidation of the intermediate 1,4-naphthol (Scheme 2.21).



Scheme 2.21 Two-step reduction/methylation approach to naphthalene ***2.35***

Tetrabutylammonium salts have been successfully employed as phase transfer catalysts for a variety of reactions involving two liquid phases. As the attempted reduction/methylation sequence involves both aqueous and organic solvent soluble reagents, the synthesis of naphthalene **2.35** via a 'one-pot' process with a phase transfer catalyst was attempted. Following the methodology of Kraus *et al.*, naphthoquinone **2.1** was successfully converted to naphthalene **2.35** in 81% yield using TBAB, dimethyl sulfate, potassium hydroxide and sodium dithionite (Scheme 2.22, route a).[33] This conversion was characterised by the appearance of a new aromatic proton peak at 6.34 ppm and the disappearance of the quinonoid singlet at 6.00 ppm in the proton NMR spectrum. In concurrent studies, we found that the reductive methylation of 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**) could also be achieved using sodium dithionite with dimethyl sulfate and potassium carbonate to give the naphthalene **2.35** in comparable yield, without the need for a phase transfer catalyst (Scheme 2.22, route b).

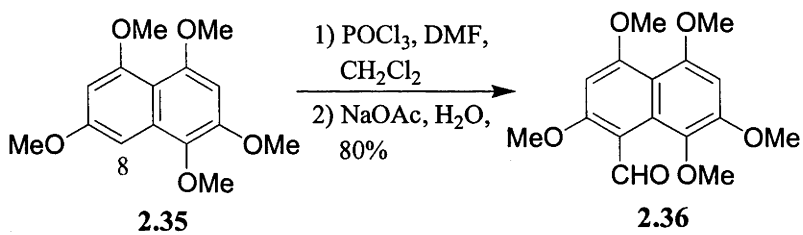


Scheme 2.22 Reductive methylation reactions of 2,5,7-trimethoxy-1,4-naphthoquinone (2.1)

Having accessed the appropriate *O*-protected naphthalene **2.35**, attempts were then made to functionalise the C8–position. It has been well documented that this position on a naphthalene system can be selectively functionalised via electrophilic aromatic substitution reactions.[34,35] We therefore attempted to formylate naphthalene **2.35** under Vilsmeier-Haack conditions to give the corresponding aromatic aldehyde **2.36** (Scheme

2.23). Initially, the formylation was attempted with 1.2 equivalents of phosphoryl chloride and dimethylformamide (DMF) in anhydrous dichloromethane. Under these conditions, however, only the starting material, naphthalene **2.35**, was recovered from the reaction mixture.The desired aldehyde **2.36** could, however, be synthesised in 80% yield by adjusting the stoichiometry of the reaction to five equivalents of phosphoryl chloride and four equivalents of DMF. The absolute requirements for the stoichiometry of the reagents cannot be easily rationalised.

The proton NMR spectrum of the aldehyde **2.36** displayed a characteristic aldehydic signal at 10.41 ppm. The disappearance of an aromatic proton at 6.34 ppm, due to the starting naphthalene **2.35**, in the proton NMR spectrum indicated that formylation had occurred at the C8-position. The formation of regioisomeric products was not evident.

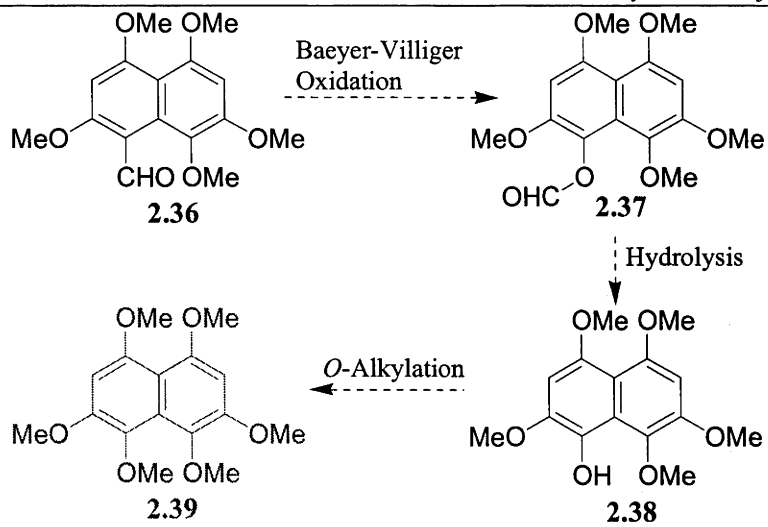


2.35 **2.36**

*Scheme 2.23 Vilsmeier-Haack formylation of naphthalene **2.35***

The Vilsmeier-Haack formylation resulted in the successful formation of a new carbon-carbon bond. As the target naphthalene system contains a carbon-oxygen bond at this position, it then remained to manipulate this functionality to give the appropriately substituted naphthalene.

In general terms, the Baeyer-Villiger oxidation reaction can be used to convert an aldehyde into a formate ester, which can then be hydrolysed to afford the corresponding alcohol. In particular, we sought to access the formate ester **2.37**, and to hydrolyse this ester to give the naphthol **2.38**. The synthesis of the symmetrically substituted naphthalene **2.39** would then be possible via the *O*-methylation of naphthol **2.38** (Scheme 2.24).

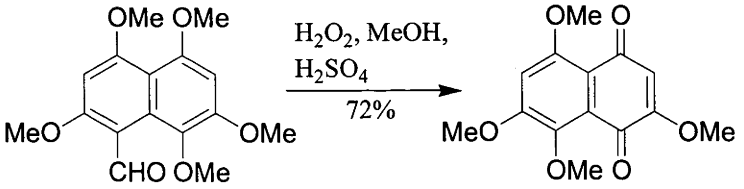


*Scheme 2.24 Proposed route to naphthalene **2.39***

The Baeyer-Villiger oxidation of aldehyde **2.36** to the formate ester **2.37**, using *m*-chloroperbenzoic acid, was consistently unsuccessful. In parallel model studies, the Baeyer-Villiger oxidation of commercially available 3,5-dimethoxybenzaldehyde using *m*-chloroperbenzoic acid was successful. Thus, the failure of the Baeyer-Villiger reaction of aldehyde **2.36** with *m*-CPBA may have been due to steric interactions between the substrate and the reagent.

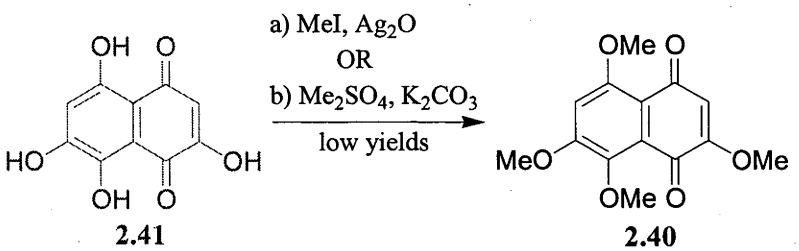
The Dakin oxidation can also be employed to convert an aromatic aldehyde into the corresponding phenol. The Dakin reaction typically involves the use of alkaline hydrogen peroxide, however, Matsumoto *et al.* have synthesised various phenols in excellent yields using acidic hydrogen peroxide.[36]

The treatment of aldehyde **2.36** with hydrogen peroxide and in acidic methanol resulted in the unexpected formation of 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**) (Scheme 2.25). Naphthoquinone **2.40** displays diagnostic singlets at 5.92 ppm and 6.72 ppm in the proton NMR spectrum due to the presence of the quinonoid and aromatic protons respectively.



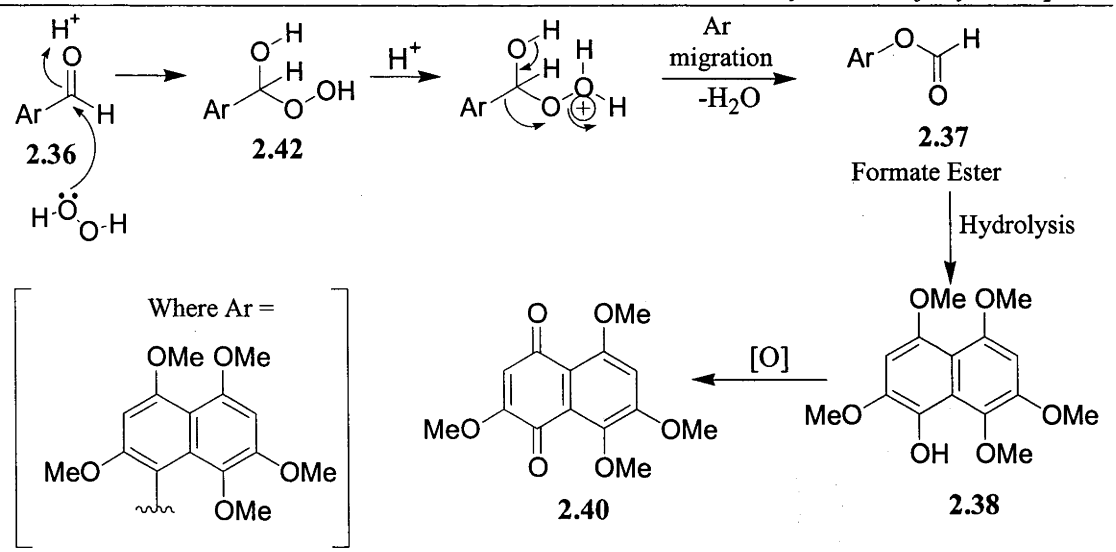
Scheme 2.25 Hydrogen peroxide/sulfuric acid oxidation of naphthalene ***2.36***

The synthesis of naphthoquinone **2.40** has previously been reported, albeit in very low yields, by Natori *et al.* through the *O*-methylation of mompain (**2.41**), a naturally occurring naphthazarin.[37] The spectroscopic and physical data determined for the unexpected product of the Dakin reaction were identical with literature data reported by Natori *et al.* for naphthoquinone **2.40** (Scheme 2.26).



Scheme 2.26 O*-Methylation of mompain (2.41) by Natori and co-workers*

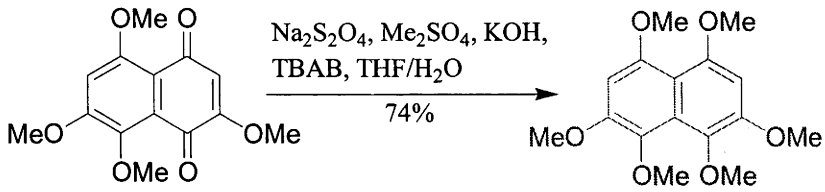
The proposed mechanism for the synthesis of naphthoquinone **2.40** is depicted in Scheme 2.27. The benzylic hydroperoxide **2.42** formed initially is protonated *in situ* and subsequent aryl migration gives the formate ester **2.37**. Hydrolysis of the formate ester **2.37**, under the acidic reaction conditions, then gives rise to the naphthol **2.38**, which is oxidised to naphthoquinone **2.40** in the presence of hydrogen peroxide (Scheme 2.27).



Scheme 2.27 Hydrogen peroxide/sulfuric acid oxidation of the aldehyde ***2.36***

The yields obtained for this reaction, and its reproducibility when performed on a large scale (>100mg), were somewhat variable. The difficulty experienced in reproducing this oxidation reaction may be due to the variable concentration of hydrogen peroxide and consequent over-oxidation of the naphthaldehyde **2.36**. It has been reported previously that oxidations of aldehydes of this type have been complicated by the formation of carboxylic acid by-products.[38] If a water-soluble aromatic carboxylic acid was formed during this reaction then this would have been inadvertently removed during the aqueous work-up step. A disappearance in the characteristic bright-red naphthoquinone colour was observed when the reaction time was increased and/or during prolonged aqueous work-up. The use of sodium perborate as an oxidant also gave the naphthoquinone **2.40** as the major product but poor mass recovery led us to abandon this approach.

With 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**) in hand, the reductive methylation conditions described previously (Scheme 2.22) were employed to successfully synthesise the key symmetrical intermediate 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) in 74% yield (Scheme 2.28).



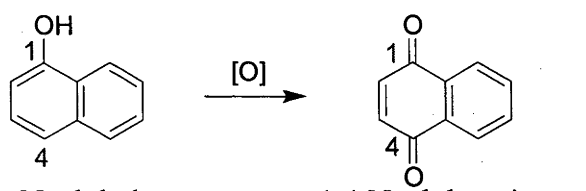
2.40 **2.39**

*Scheme 2.28 Reductive methylation of 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**)*

The simplicity of the proton NMR spectrum of naphthalene **2.39** attests to its symmetrical nature. Only four distinct chemical environments were observed in the proton NMR spectrum, that is, a 2H aromatic signal is observed at 6.54 ppm (2H) and three 6H singlet resonances arise from the three methoxy proton environments.

*2.6 Alternative Approach to 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**)*

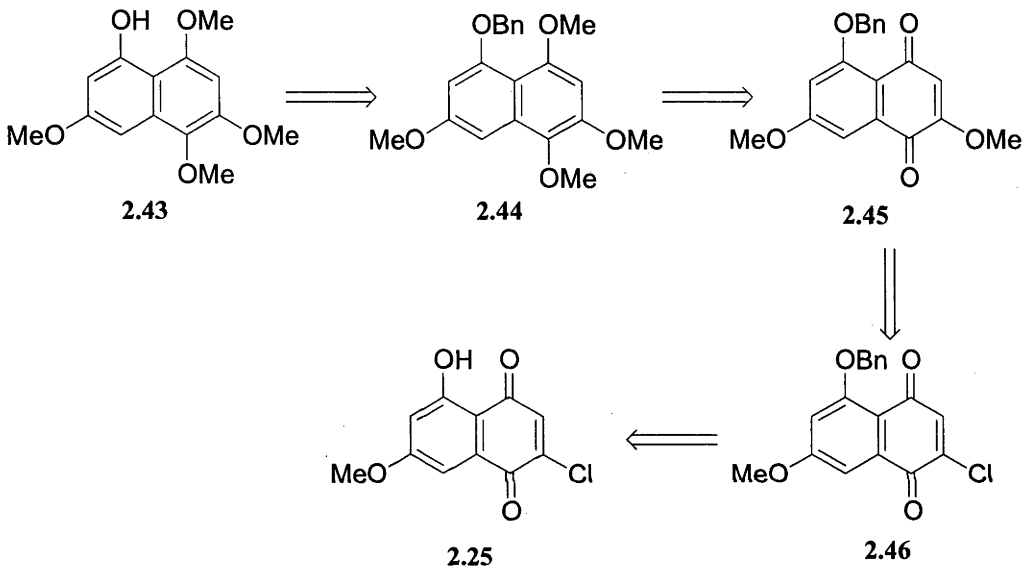
As naphthoquinone **2.40** could only be accessed in low yield, we wished to explore alternative approaches to this naphthoquinone so as to increase the efficiency of our synthetic route. As discussed in Chapter One, the oxidation of an appropriately substituted naphthol can give rise to the formation of the corresponding 1,4-naphthoquinone in good yield (Scheme 2.29).[39] Such an oxidation approach is advantageous as it does not require 1,4-difunctionalised precursors.



Naphthol 1,4-Naphthoquinone

Scheme 2.29 Naphthol oxidation approach to the synthesis of 1,4-naphthoquinones

A synthetic route to the appropriately substituted naphthol **2.43** was therefore designed. The selective, hydrogenolytic deprotection of an *O*-benzyl ether **2.44** should give access to the desired naphthol **2.43**. This *O*-benzyl ether **2.44** may in turn be synthesised via the reductive methylation of naphthoquinone **2.45**. It was anticipated that naphthoquinone **2.45** can be formed through a nucleophilic displacement reaction analogous to that described in Section 2.4. The appropriate 2-chloro-1,4-naphthoquinone **2.46** should be accessible via the *O*-benzylation of the Diels-Alder product, naphthoquinone **2.25**, the synthesis of which has been described in Section 2.4.2.

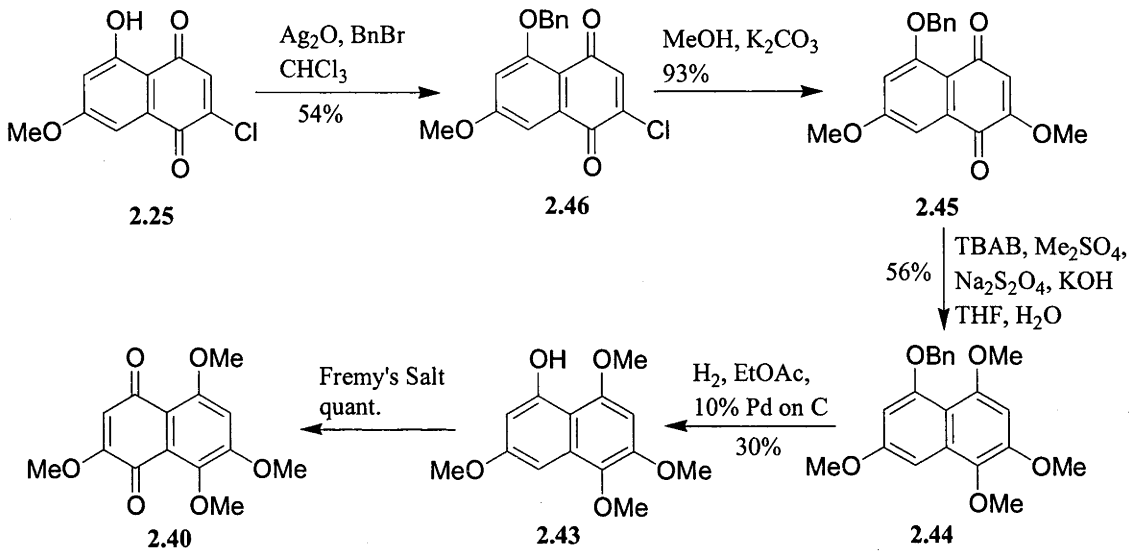


Scheme 2.30 Retrosynthetic approach to naphthalene 2.43

The first step in this synthetic sequence involved *O*-benzylation of the Diels-Alder adduct naphthoquinone **2.25**. This proceeded smoothly to give the *O*-benzylnaphthoquinone **2.46** in moderate yield. The naphthoquinone **2.46** had characteristic benzylic signals in the proton NMR spectrum as well as additional aromatic peaks attributed to the benzylic functionality (Scheme 2.31).

The naphthoquinone **2.46** was then treated with potassium carbonate in methanol to afford dimethoxy-1,4-naphthoquinone **2.45** with excellent regioselectivity and yield. The naphthoquinone **2.45** was reductively methylated, and the resultant naphthalene **2.44** subjected to hydrogenolysis using 10% palladium on carbon catalyst under a hydrogen atmosphere to give the naphthol **2.43** in poor yield. Oxidation of the naphthol **2.43** with commercially available Fremy's salt then proceeded to give the desired

naphthoquinone **2.40** in quantitative yield (Scheme 2.31). This material was spectroscopically identical to that synthesised previously.

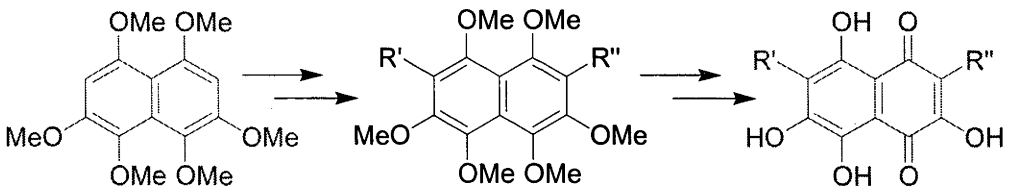


*Scheme 2.31 Alternative synthetic approach to naphthoquinone **2.40***

Thus, to date, we have developed two viable synthetic routes to naphthoquinone **2.40**, which can be easily converted into naphthalene **2.39**, through methodology discussed previously. Both synthetic routes comprise of five steps from the naphthoquinone **2.25** to the tetramethoxy naphthoquinone **2.40**. The first synthesis of naphthoquinone **2.40**, however, proved to be the more efficient route because of the superior yields obtained. It then remained to further elaborate on the naphthoquinone framework so as to access the appropriately substituted naturally occurring naphthazarins.

2.7 The Synthesis of Naturally Occurring Naphthazarins

Following the successful synthesis of naphthalene **2.39**, the appropriately *C*-alkylated naphthalene systems were the next synthetic targets en route to the desired naturally occurring naphthazarins.



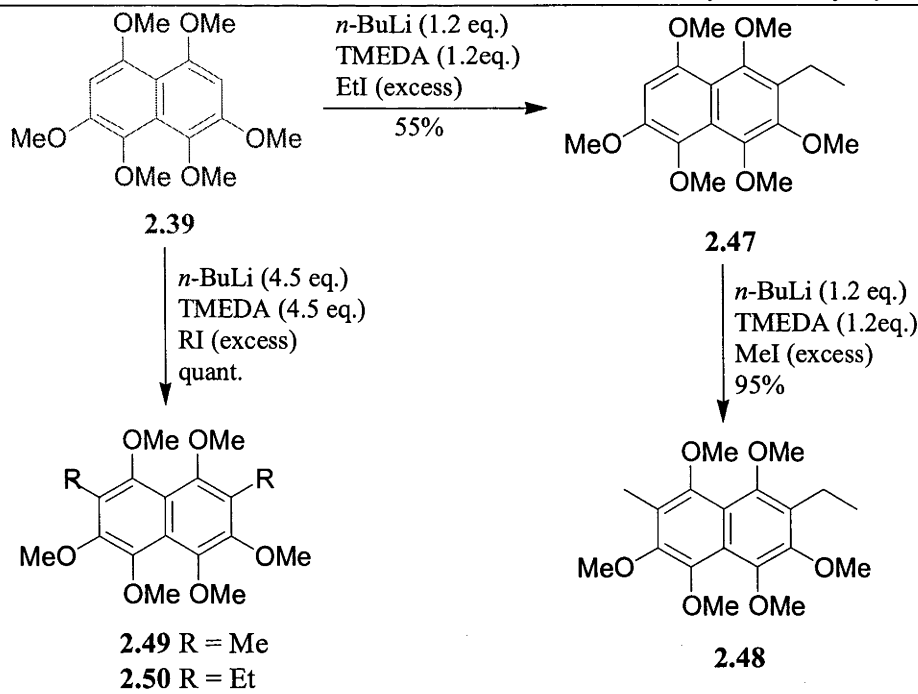
Naphthalene **2.39** Naturally Occurring Naphthazarins

Scheme 3.32 Synthetic approach to naturally occurring naphthazarins

*2.7.1 The Installation of Functionality on Naphthalene **2.39** via C-Alkylation*

Our strategy for the alkylation of the aromatic naphthalene core involved metallation using an organolithium base, followed by alkylation of the aryl lithium thereby generated with an alkyl halide. Initial attempts to *C*-alkylate 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) using a slight excess of *n*-butyl lithium (*n*-BuLi) and a large excess of ethyl iodide failed to yield any of the desired product and only starting material was recovered from the reaction mixture. When other bases such as *tert*-butyl lithium and the 'superbase' LiCKOR (prepared *in situ*) were employed, the alkylation reaction was also unsuccessful.[40-43]

The use of basic additives in lithiation reactions has been well documented in the literature. Snieckus *et al.* have successfully used tetramethylethylenediamine (TMEDA) to de-aggregate *n*-butyl lithium hexamers, which increases the basicity of *n*-butyl lithium.[44,45] When 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) was treated with 1.2 equivalents of *n*-butyl lithium and 1.2 equivalents of TMEDA, followed by a large excess of ethyl iodide (Scheme 2.33), the monoethylated naphthalene **2.47** was isolated in 55% yield, together with 23% of the dialkylated material and recovered starting material. The monoethylated naphthalene **2.47** displayed a characteristic proton singlet at 6.62 ppm in the aromatic region of the proton NMR spectrum. The naphthalene **2.47** can be further *C*-alkylated in excellent yield under comparable reaction conditions using methyl iodide to give the mixed dialkylated naphthalene **2.48**. The proton NMR spectrum of the naphthalene **2.48** lacks the aromatic hydrogen signal and typical *C*-methyl and *C*-ethyl resonances, as well as *O*-methyl signals, are observed.



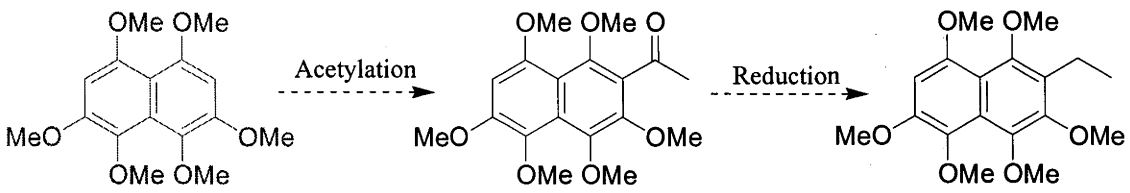
Scheme 2.33 Alkylation of the symmetrical intermediate ***2.39***

When 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) was treated with 4.5 equivalents of both *n*-butyl lithium and TMEDA, followed by a large excess of methyl iodide, the symmetrically substituted 1,2,4,5,7,8-hexamethoxy-3,6-dimethylnaphthalene (**2.49**) was obtained in quantitative yield. The dimethyl naphthalene **2.49** was characterised by a singlet in the proton NMR spectrum at 2.33 ppm (6H) corresponding to the two equivalent aromatic methyl resonances and by three singlets (18H) due to the three distinct *O*-methyl environments. The corresponding ethyl derivative, diethyl naphthalene **2.50**, was likewise synthesised by the analogous addition of ethyl iodide. Naphthalene **2.50** was spectroscopically identical to the dialkylated by-product isolated during the formation of naphthalene **2.47**.

2.7.2 Attempted C-Acetylation of Naphthalene ***2.39***

Although the *C*-alkylation strategy above was successful, and allows for the installation of the alkyl substituents present in the target naphthazarins, the inherent competition between mono- and dialkylation leads to moderate yields of the desired naphthalene **2.47** at best. An alternative synthesis of naphthalene **2.47** may involve the acetylation of the naphthalene core, followed by the reduction of the acetyl group to the appropriate ethyl substituent. As the first step would involve the Friedel-Crafts acetylation of

naphthalene **2.39**, multiple acylation is unlikely due to the deactivation of the subsequent aromatic ring to electrophilic aromatic substitution.[46]



2.39 **2.47**

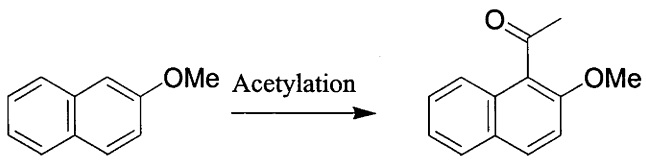
*Scheme 2.34 Proposed approach to naphthalene **2.47***

Initial attempts to perform a Friedel-Crafts acetylation on the symmetrical naphthalene **2.39** involved the use of acetic anhydride with a catalytic amount of perchloric acid. A complex reaction mixture was obtained upon work-up, however, despite adjusting the quantities of perchloric acid employed (Entry 1, Table 2.2).

Jaxa-Chamiec *et al.* have reported the successful acetylation of a number of methoxy-substituted aromatic compounds using aluminium trichloride as the catalyst and acyl chlorides as the acylating agents.[47] Their work also outlined the scope for *in situ* reduction of the acyl functionality to the corresponding alkyl group via the addition of triethylsilane to the reaction mixture. The reaction of naphthalene **2.39** with anhydrous aluminium trichloride and freshly distilled acetyl chloride in dry dichloromethane at room temperature proceeded rapidly with immediate consumption of the starting material as monitored by TLC (Entry 2, Table 2.2). Upon work-up, an as yet uncharacterised orange compound was obtained in low yield. This compound displayed typical quinone signals in the proton NMR and may have arisen from the cleavage of methyl ethers in the presence of the strong Lewis acid, followed by aerial oxidation.

The acetylation of 2-methoxynaphthalene (**2.51**) has been reported by Zemina *et al.* using iron trichloride and acetyl chloride. We were able to successfully follow this methodology to acetylate the naphthalene **2.51** to give ketone **2.52** in good yield (Scheme 2.35).[48] Disappointingly, however, 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) did not react under analogous reaction conditions (Table 2.2, Entry 3).

Person *et al.* have extensively studied the use of catalysts in Friedel-Crafts acylations.[49] In particular, a range of aromatic alkyl ethers have been successfully acetylated with acetic anhydride in the presence of small amounts of iodine at elevated temperatures. In model studies, we were able to acetylate 2-methoxynaphthalene (**2.51**) to give 1-acetyl-2-methoxynaphthalene (**2.52**) in excellent yield under these conditions. The treatment of 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) under analogous reaction conditions resulted in the formation of a complex reaction mixture (Entry 4, Table 2.2).



2.51 **2.52**

Scheme 2.35 Model acetylation reactions

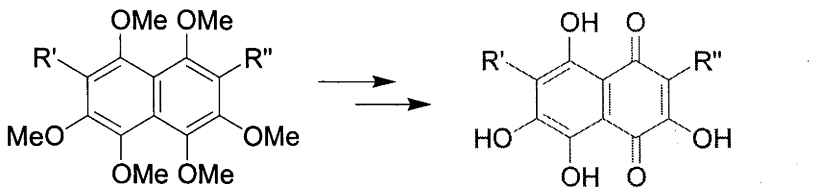
An alternative approach to *C*-acylation is to generate the appropriate aryl lithio species *in situ* and quench the anion with a suitable electrophile. When we treated naphthalene **2.39** with *n*-butyl lithium at -78°C followed by the addition of *N,N*-dimethylacetamide (DMA) a complex reaction mixture was obtained (Entry 5, Table 2.2). Given the difficulties thereby encountered, the approach involving the selective installation of *C*-acetyl functionality on the symmetrical intermediate **2.39** was abandoned.

*Table 2.2 Attempted Acetylation of Naphthalene **2.39***

Entry	**Reagent(s)**	**Temperature, Time**	**Observation**
1	Ac_2O, $HClO_4$	RT, 1.5h	Complex reaction mixture
2	$AlCl_3$, AcCl, DCM	RT, 2 min	Complex reaction mixture
3	$FeCl_3$, Ac_2O, CH_2ClCH_2Cl	70°C, 2 days	No reaction
4	I_2, Ac_2O	Reflux, 3h	Complex reaction mixture
5	*n*-BuLi, TMEDA, THF, DMA	-78°C, 1h	Complex reaction mixture

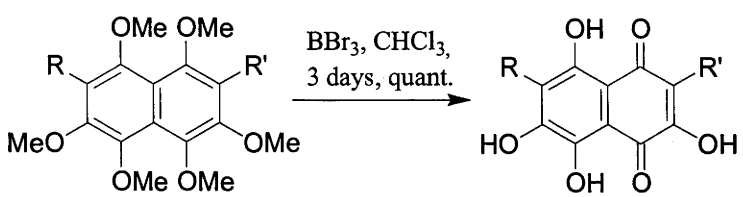
2.7.3 Oxidation of the Functionalised Naphthalenes

Following the synthesis of the alkylated naphthalenes **2.47-2.50**, the naturally occurring naphthazarins were regarded as the next synthetic targets (Scheme 2.36). Methyl ether cleavage followed by oxidation should afford the appropriately substituted naphthazarins.[50,51]



Scheme 2.36 Proposed deprotection/oxidation to give naphthazarins

3-Ethyl-1,2,4,5,7,8-hexamethoxynaphthalene (**2.47**), 3-ethyl-1,2,4,5,7,8-hexamethoxy-6-methylnaphthalene (**2.48**), 1,2,4,5,7,8-hexamethoxy-3,6-dimethylnaphthalene (**2.49**) and 1,2,4,5,7,8-hexamethoxy-3,6-diethylnaphthalene (**2.50**) were treated in turn with boron tribromide over three days. Under these conditions, the methyl ethers were cleaved and *in situ* aerial oxidation ensued to give the deep purple/red naphthazarins aureoquinone (**1.14**), 3-ethyl-2,7-dihydroxy-1,4-naphthazarin (**1.15**), 3(6)-ethyl-2,7-dihydroxy-6(3)-methyl-1,4-naphthazarin (**1.16**), and 3,6-diethyl-2,7-dihydroxy-1,4-naphthazarin (**2.53**) in quantitative yield (Scheme 2.37). The products were purified by recrystallisation from ethanol and were found to be spectroscopically identical to the natural products reported in the literature. The tautomeric structure of naphthazarin **1.15** is based on the assignment of Moore and co-workers. We have therefore successfully developed a convenient and versatile route to highly functionalised naphthazarins, including several natural products.



2.47 R = H; R' = Et
2.48 R = Me; R' = Et
2.49 R = R' = Me
2.50 R = R' = Et

1.15 R = H; R' = Et
1.16 R = Me; R' = Et or R = Et; R' = Me
1.14 R = R' = Me
2.53 R = R' = Et

Scheme 2.37 Synthesis of functionalised naphthazarins

2.7.4 Confirmation of the Boryquinone Structure

One of the primary objectives of this work was to confirm the structure of boryquinone, isolated by Huneck *et al.* from lichen cultures of *Cladonia boryi* (Tuck.) Cladoniaceae. We were able to compare our synthetically derived 3(6)-ethyl-2,7-dihydroxy-6(3)-methyl-1,4-naphthazarin (**1.16**) with the bright purple boryquinone provided by the authors. The two compounds were identical as shown by HPLC-UV/Vis analysis and spectroscopic data (Figure 2.3). We were also interested to note that boryquinone **(1.16)** was present in extracts of the lichen *Leconora hybocarpa* (Tuck.) Brodo, from which hybocarpone **(1.25)** was extracted.

The studies thus far have therefore culminated in the synthesis of three naturally occurring naphthazarins: boryquinone **(1.16)**, aureoquinone **(1.14)** and 3-ethyl-2,7-dihydroxynaphthazarin **(1.15)**.

2.8 The Formal Total Synthesis of Hybocarpone (1.25)

In 2001, Nicolaou and Gray reported the first total synthesis of racemic hybocarpone **(1.25)** from the hydroxy naphthoquinone **1.63**.[52] Given our interest in accessing significant quantities of hybocarpone **(1.25)** for biological testing, we also wished to synthesise the naphthoquinone **1.63**.

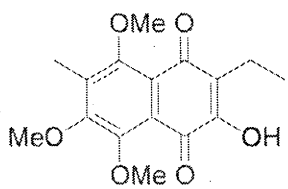


*Figure 2.5 2-Hydroxy-1,4-naphthoquinone **1.63***

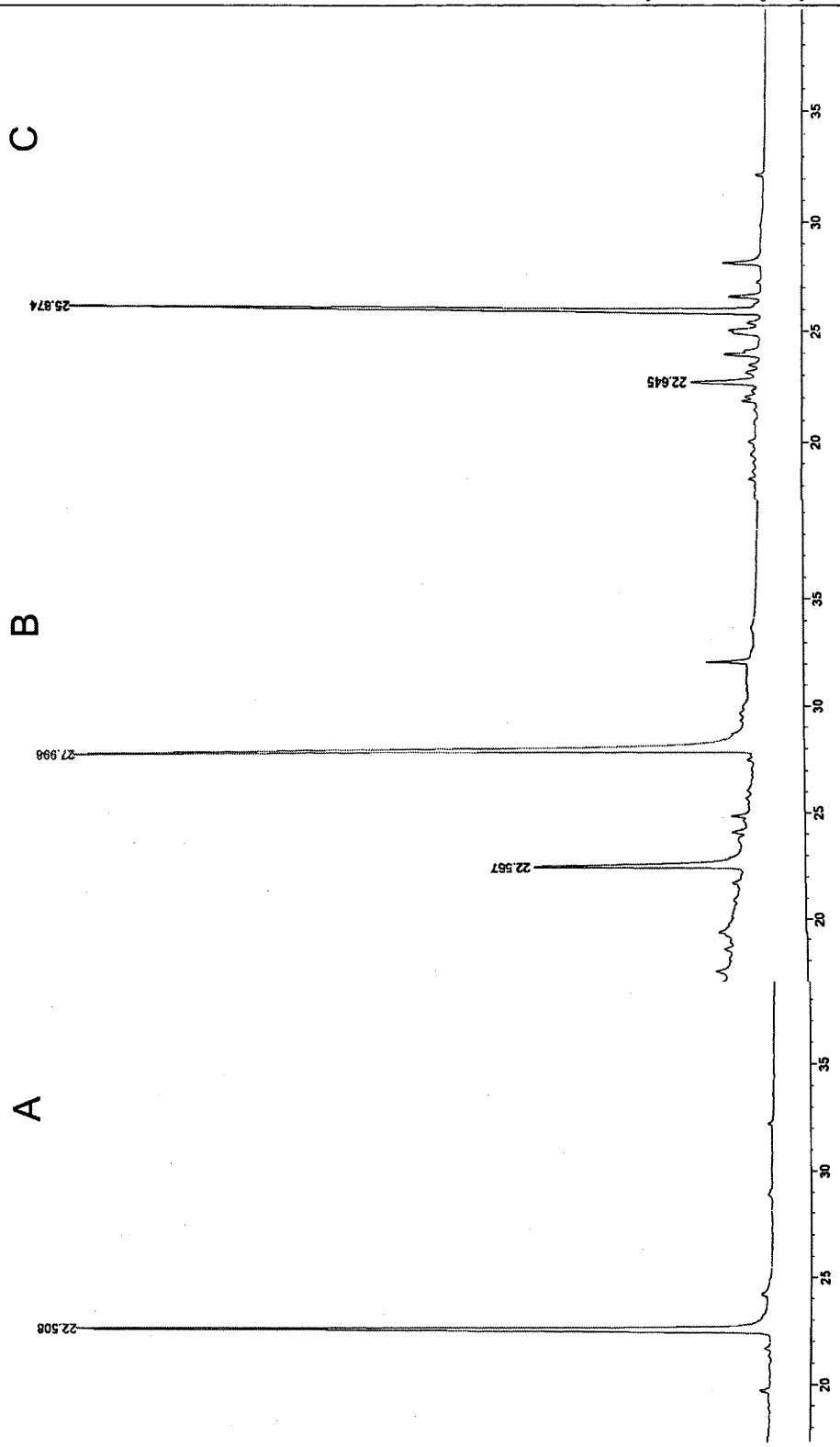


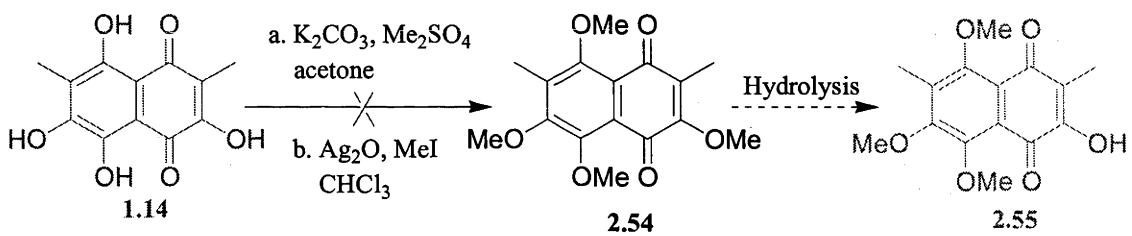
*Figure 2.3 HPLC traces (retention time 20 min to 35 min shown) of: a) synthetic boryquinone (**1.16**); b) extracts of the lichen* Cladonia boryi*; and c) extracts of the lichen* Leconora hybocarpa. *(Boryquinone (**1.16**) is shown in magenta, usnic acid, a common lichen metabolite, is shown in green and hybocarpone (**1.25**) is shown in red).*

2.8.1 Attempted O*-Alkylation of Naphthazarins*

We initially sought to develop a synthetic route to the desired hydroxy naphthoquinone **1.63**, and analogues thereof, from the naphthazarins synthesised previously (Section

2.7.3), given the successful conversion of mompain (**2.41**) into the corresponding *O*-methyl ether, naphthoquinone **2.40** (Scheme 2.26). A symmetrical system was thought to provide the simplest model for studying this approach and so aureoquinone (**1.14**) was employed as a model system. We envisaged that *O*-alkylation of the phenolic groups of the naphthazarin **1.14** would give rise to tetramethoxy naphthoquinones, such as **2.54,** which could be converted to the corresponding hydroxy naphthoquinone (**2.55**) through acid hydrolysis (Scheme 2.38).

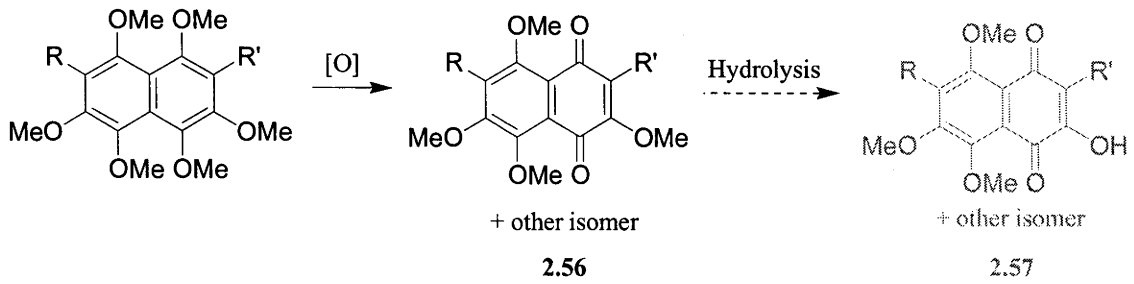
When aureoquinone (**1.14**) was treated with potassium carbonate and dimethyl sulfate in refluxing acetone a complex mixture of products was obtained. When silver(I) oxide and methyl iodide were used instead, a complex mixture of products was again obtained (Scheme 2.38). Given these disappointing results, alternative means of accessing the desired 2-hydroxyl,4-naphthoquinone **1.63** were sought.



Scheme 2.38 Attempted synthesis of 2-hydroxy-1,4-naphthoquinone ***2.55***

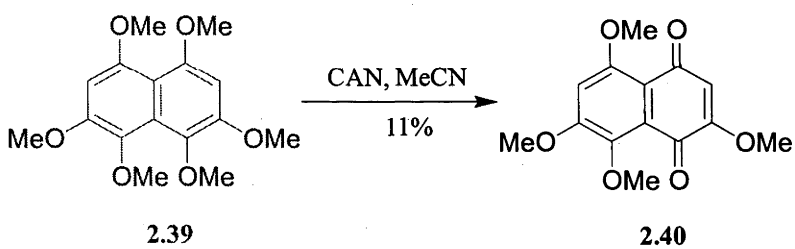
2.8.2 Non-selective Oxidative Approach towards Naphthoquinone ***1.63***

An alternative approach involving the oxidation of an appropriately substituted naphthalene, such as those synthesised previously (Section 2.7.1), to a naphthoquinone **2.56** through oxidative methyl ether cleavage was envisaged. From intermediates of this type it should be possible to access the desired 2-hydroxy-1,4-naphthoquinone **2.57** through hydrolysis. This approach is not, however, regioselective and the oxidation of unsymmetrically substituted naphthalenes would therefore result in the formation of a mixture of regioisomers (Scheme 2.39).



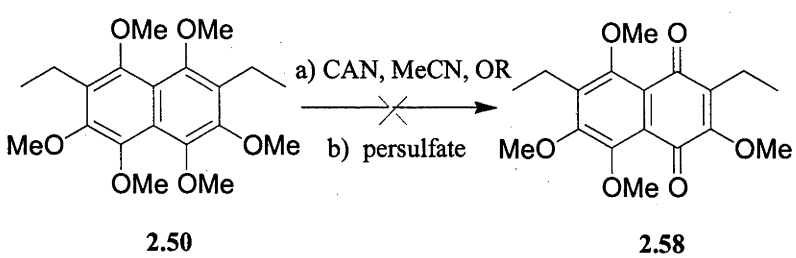
Scheme 2.39 The oxidation/hydrolysis approach to hydroxy naphthoquinones

Our initial investigations utilised the symmetrically substituted (that is R = R') naphthalenes synthesised previously, so as to remove issues of selectivity. The symmetrically substituted 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) was treated with ceric ammonium nitrate (CAN) in acetonitrile to give the corresponding naphthoquinone **2.40** in an unoptimised yield of 11%. The product isolated was identical to the naphthoquinone **2.40** synthesised previously and was readily identified by the presence of singlets at 5.92 ppm and 6.72 ppm, along with four distinct methyl ether signals, in the proton NMR spectrum.



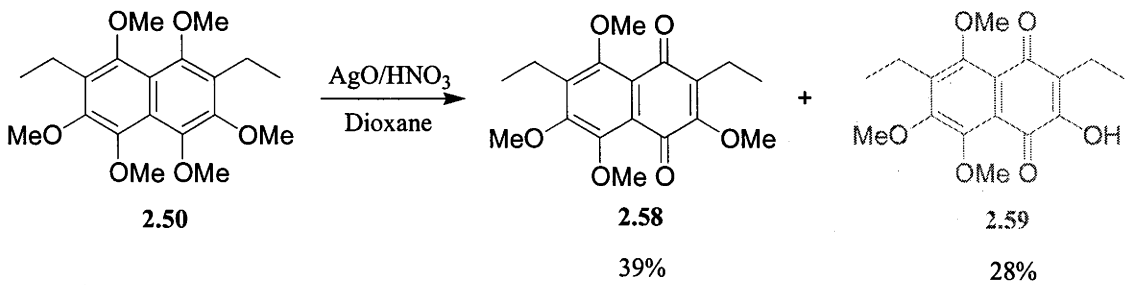
*Scheme 2.40 Successful oxidation of symmetrically substituted system **2.39***

When the dialkylated naphthalene **2.50** was treated with CAN, however, a complex reaction mixture was obtained. The use of potassium persulfate as an oxidising agent also resulted in a complex reaction mixture (Scheme 2.41).



*Scheme 2.41 Attempted oxidations of naphthalene **2.50***

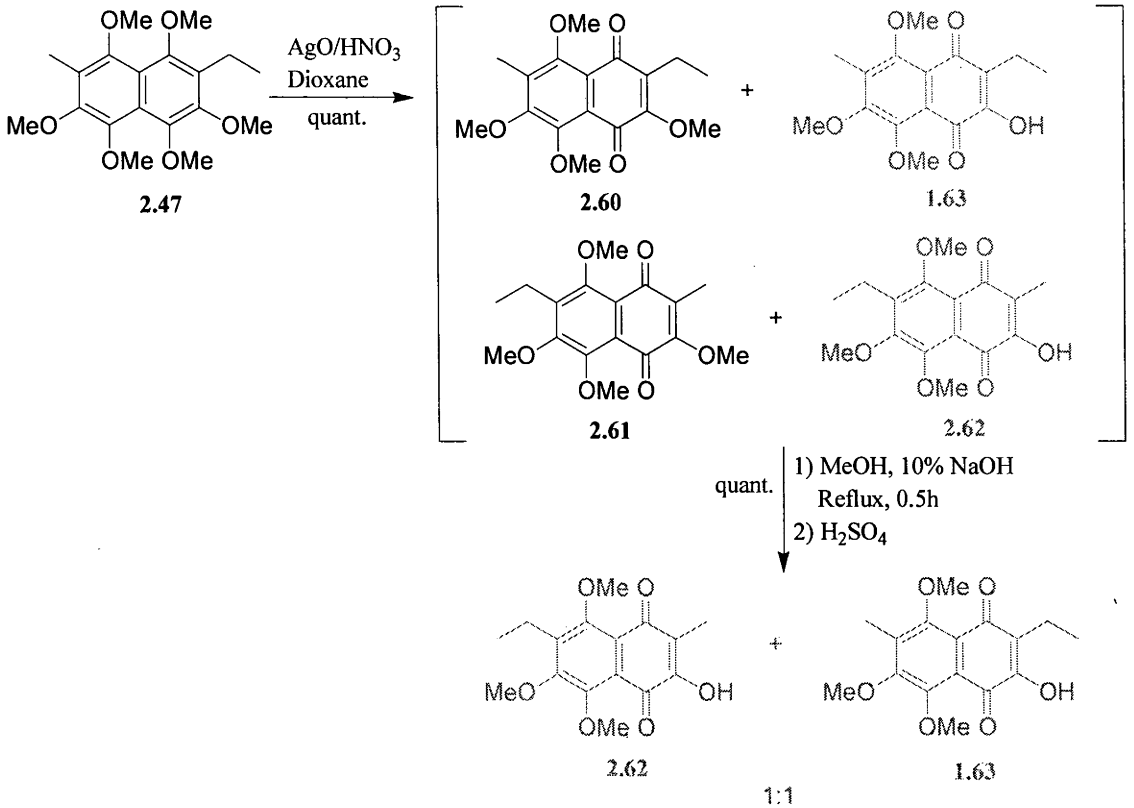
When the oxidation reaction was attempted utilising the classic Rapoport oxidation conditions with freshly prepared silver(II) oxide and nitric acid,[53] the desired bright orange naphthoquinone **2.58** was isolated in 39% yield after chromatography, along with 28% of the yellow hydroxy napthoquinone **2.59**, presumably formed by the *in situ* acid catalysed hydrolysis of naphthoquinone **2.58** (Scheme 2.42).



2.50 **2.58** 39% **2.59** 28%

*Scheme 2.42 Silver oxide/ nitric acid oxidation of symmetrically dialkylated naphthalene **2.50***

Following this promising result, we then attempted an analogous reaction using the unsymmetrical dialkylated naphthalene **2.47**. The oxidation proceeded smoothly to give four products, which were spectroscopically identified as the two isomeric naphthoquinones **2.60** and **2.61** and the corresponding hydrolysis products **2.62** and **1.63**. The product mixture was then hydrolysed under acidic conditions to give the 2-hydroxy naphthoquinones **2.62** and **1.63** (Scheme 2.43).



Scheme 2.43 Oxidation of non-symmetrically substituted naphthalene ***2.47***

Unfortunately, these isomers could not be separated chromatographically. Examination of the proton NMR spectrum of the reaction mixture indicated that the desired naphthoquinone **1.63** was present, by comparison with the spectroscopic data reported for this compound by Nicolaou and Gray.

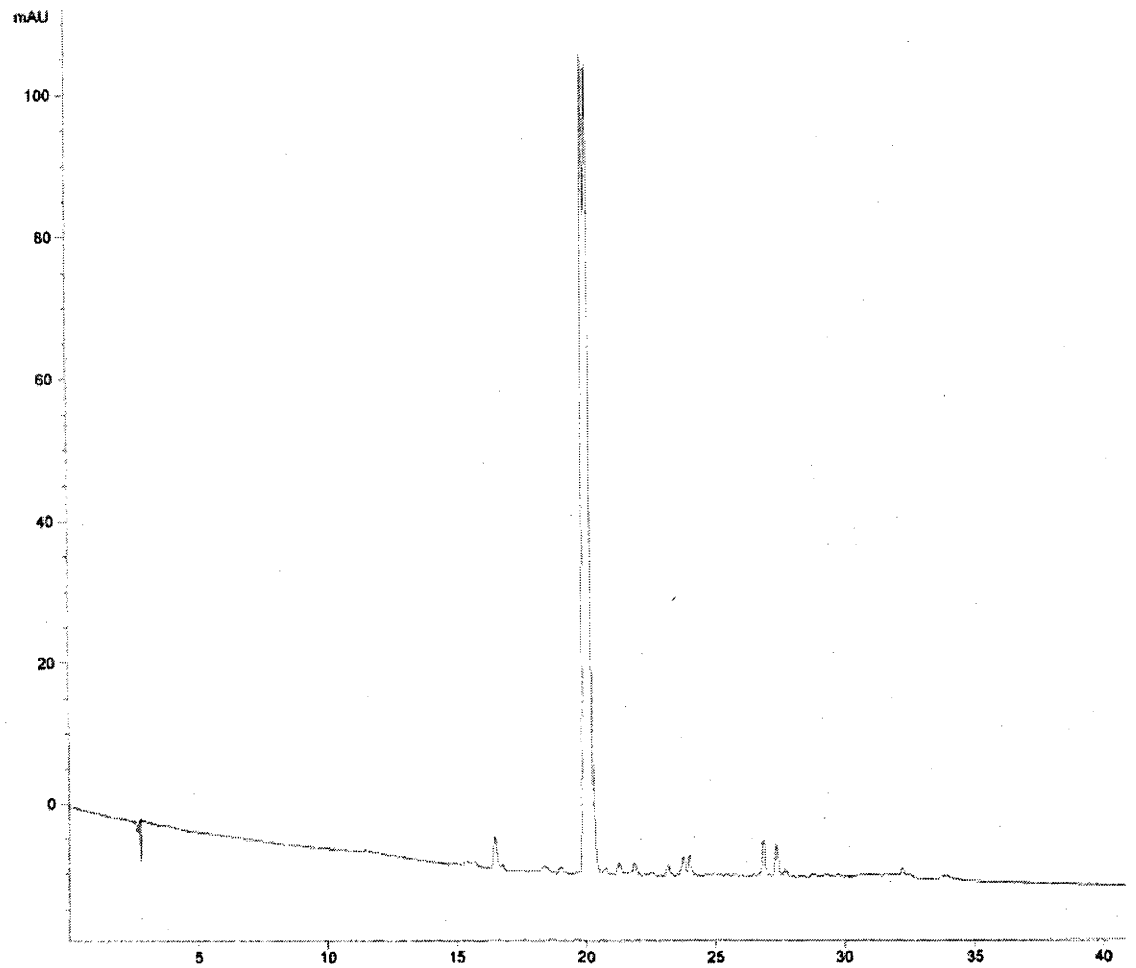


Figure 2.5 Absorbance (mAU) versus retention time (min): regioisomeric mixture of naphthoquinones ***2.62/1.63***

While this constituted a formal total synthesis of hybocarpone (**1.25**), we were disappointed by our inability to separate the target hydroxy naphthoquinone **1.63** from its regioisomer. Rather than pursue the chromatographic separation of the methoxy naphthoquinones **2.60** and **2.61** we chose to develop a selective synthesis of 2-hydroxy-1,4-naphthoquinones, and this approach will be discussed in Chapter Three.

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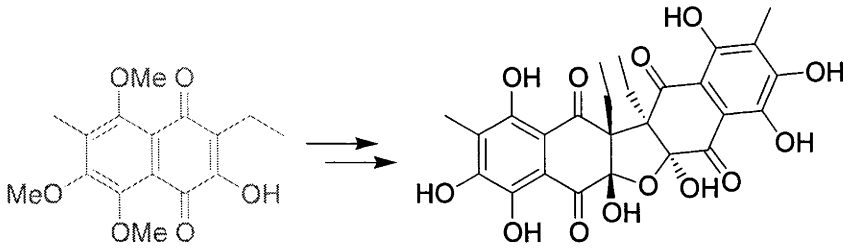
Chapter Three:

The Formal Total Synthesis of Hybocarpone

3.1 Introduction

Given our interest in accessing appreciable quantities of hybocarpone (**1.25**), we aimed to synthesise 2-hydroxy-1,4-naphthoquinone **1.63**, the precursor utilised by Nicolaou and Gray in their total synthesis of hybocarpone (**1.25**) (Scheme 3.1).[1]



1.63 **1.25**

Hybocarpone

Only relative stereochemistry is known.

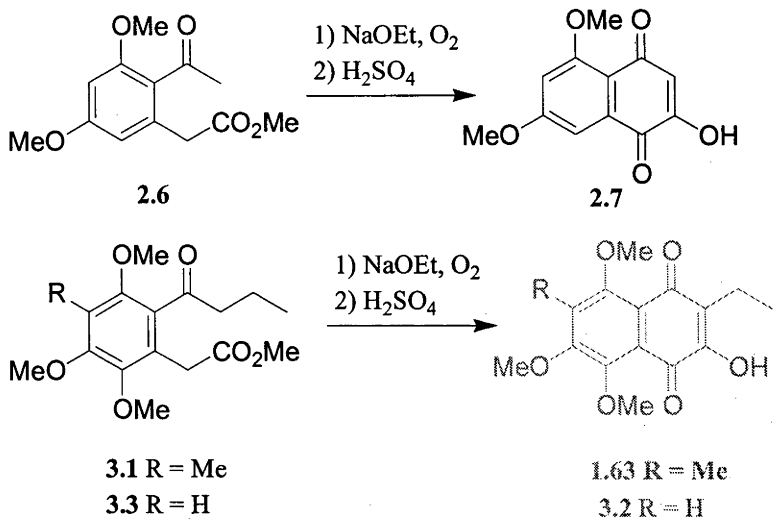
Scheme 3.1 Nicolaou et al. *synthesis of hybocarpone (1.25)*

As outlined in the previous chapter, attempts to synthesise this target compound via the non-selective oxidation of naphthalene **2.47** resulted in an inseparable mixture of regioisomers (Section 2.8.2). The development of a selective, expedient synthetic route to 2-hydroxy-1,4-naphthoquinone **1.63** was therefore highly desirable.

3.2 Selective Synthetic Approach to the Synthesis of 2-Hydroxy-1,4-naphthoquinones

The studies of Bycroft *et al.* involved the regioselective formation of 2-hydroxy-1,4-naphthoquinone **2.7** via the Claisen condensation and aerial oxidation of the appropriate precursor, ketone **2.6** (Scheme 3.2).[2] Several other groups have also successfully applied this protocol towards the synthesis of functionalised naphthoquinones.[3-5] Synthetic access to the 2-hydroxy-1,4-naphthoquinone **1.63** should therefore be possible from ketone **3.1**, through an analogous approach.

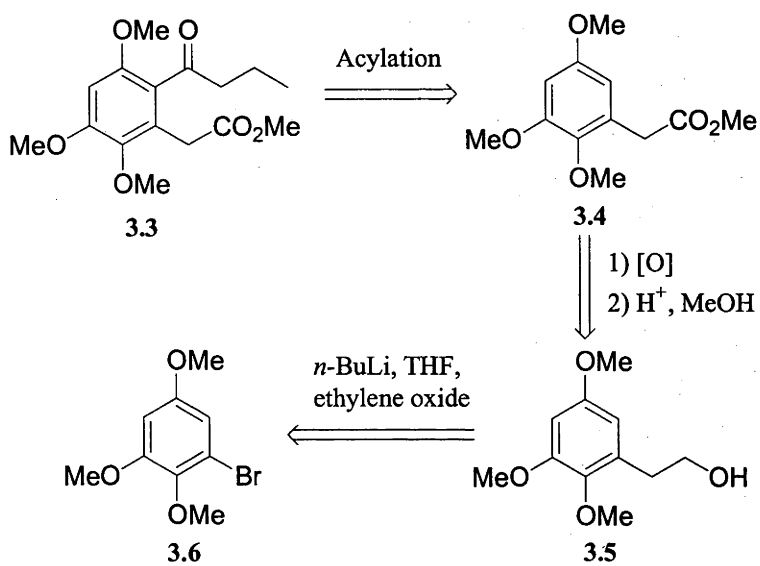
Our initial investigations focused on the synthesis of 2-hydroxy-3-ethyl-5,7,8-trimethoxy-1,4-naphthoquinone (**3.2**), as part of our strategy to access synthetic analogues of hybocarpone (**1.25**), as well as to examine the feasibility of such an approach to highly oxygenated precursors.



Scheme 3.2 Claisen condensation/oxidation approach to naphthoquinones

3.2.1 Retrosynthetic Analysis

A retrosynthetic analysis of the key ketone **3.3** reveals that the desired compound may be formed through a regioselective Friedel-Crafts type acylation of the appropriately functionalised methyl ester **3.4**. We designed a synthesis of methyl ester **3.4** based on the oxidation of the appropriate primary alcohol **3.5**, followed by esterification. The installation of the two-carbon unit present in the alcohol **3.5** may be achieved through lithiation of a halobenzene derivative, such as the known compound bromobenzene **3.6**, followed by treatment with ethylene oxide (Scheme 3.3).

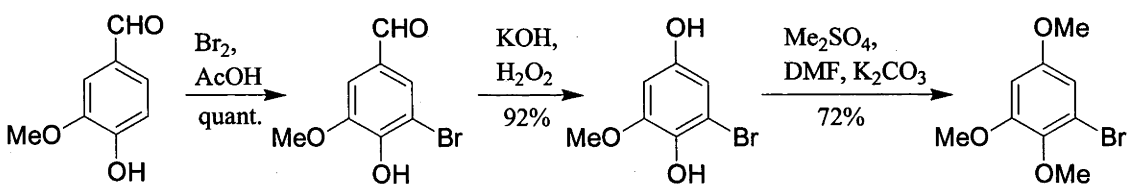


*Scheme 3.3 Retrosynthetic analysis of desired ketone **3.3***

3.2.2 The Synthesis of Bromobenzene **3.6**

Several synthetic routes to the desired bromobenzene **3.6** have been reported in the literature.[6] Of these, Dorn *et al.* have reported the most direct synthesis of bromobenzene **3.6**, from the inexpensive, commercially available *p*-vanillin (**2.14**), and we therefore utilised their procedure for the synthesis of bromobenzene **3.6**.[7]

The first step in the synthetic sequence involved the quantitative formation of bromobenzaldehyde **3.7** following the treatment of *p*-vanillin (**2.14**) with bromine in glacial acetic acid. The benzaldehyde **3.7** was subsequently oxidised under Dakin conditions to give the corresponding hydroquinone **3.8** after acidic work-up. In this manner, the desired oxygenation pattern on the aromatic ring was achieved. In our hands, however, the large-scale formation of hydroquinone **3.8** proceeded with variable yields and over-oxidation was frequently observed. The hydroquinone **3.8** was then efficiently converted into the tri-*O*-methyl ether derivative **3.6** in good yield (Scheme 3.4). As the Dakin oxidation step was problematic, large quantities of the bromobenzene **3.6** could not be obtained via this synthetic pathway. Consequently, an alternative route to the synthesis of bromobenzene **3.6** was examined.



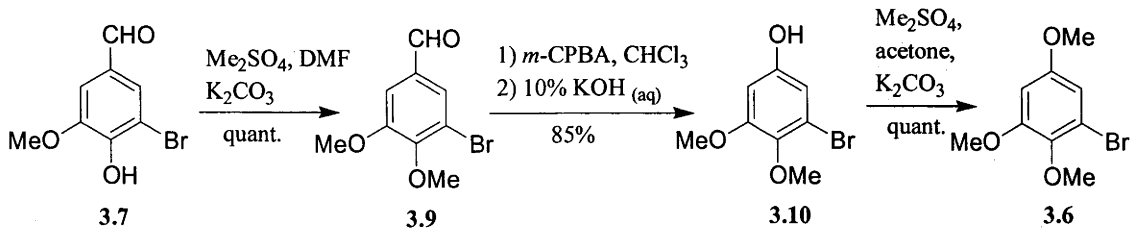
2.14 **3.7** **3.8** **3.6**

Scheme 3.4 Dorn et al. *synthesis of bromobenzene* **3.6**

Sanchez and co-workers reported the synthesis of bromobenzene **3.6** using a Baeyer-Villiger oxidation of dimethoxybenzaldehyde **3.9**.[8] We were able to access the required aldehyde **3.9** in quantitative yield through *O*-methylation of the phenol **3.7** already in hand (Scheme 3.5).

Following the procedure of Sanchez *et al.*, the oxidation of aldehyde **3.9** proceeded smoothly to give phenol the **3.10** in 85% yield. The subsequent *O*-methylation of phenol **3.10** then gave 1-bromo-2,3,5-trimethoxybenzene (**3.6**) in quantitative yield (Scheme 3.5). In contrast to the procedure developed by Dorn *et al.*, this synthetic route enabled

access to 1-bromo-2,3,5-trimethoxybenzene (**3.6**) in 83% overall yield after four synthetic steps from aldehyde **3.7**.

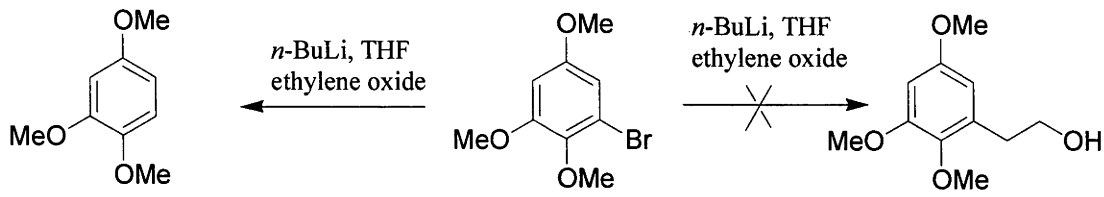


*Scheme 3.5 Alternative synthesis of the desired bromobenzene **3.6***

3.2.3 Attempted Synthesis of Alcohol 3.5

Following the synthesis of bromobenzene **3.6** attempts were made to install the two-carbon unit of the alcohol **3.5** through lithiation of bromobenzene **3.6** and reaction of the subsequent anion with ethylene oxide (Scheme 3.6). The bromobenzene **3.6** was initially treated with one equivalent of *n*-butyl lithium in THF prior to the addition of 1.5 equivalents of neat ethylene oxide via a pre-cooled syringe.[9] Disappointingly, only 1,2,4-trimethoxybenzene (**3.11**) was isolated from the reaction mixture and when the reaction was repeated using a large excess of ethylene oxide, a similar outcome was obtained. The isolation of 1,2,4-trimethoxybenzene (**3.11**) from these studies indicates that the halogen-metal exchange reaction is successful but that the anion generated does not react with ethylene oxide.

According to the general investigations of Eis *et al.*, boron trifluoride diethyl etherate promotes the nucleophilic addition of strongly basic aryl lithium species to electrophilic oxiranes.[10] The reason for this observed enhancement is thought to be complex but may involve the coordination of the Lewis acid to the cyclic ether prior to reaction with the lithio species, thereby increasing the electrophilicity of the oxirane. When one equivalent boron trifluoride diethyl etherate was used, however, only 1,2,4-trimethoxybenzene (**3.11**) was isolated. The reaction was repeated at –20°C but only a complex mixture of products was obtained. These experimental difficulties led us to abandon this approach towards the synthesis of methyl ester **3.4**.



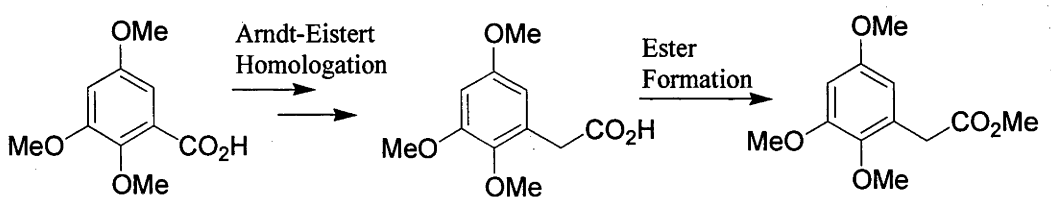
3.11 **3.6** **3.5**

*Scheme 3.6 Attempted synthesis of alcohol **3.5***

*3.2.4 The Synthesis of Methyl Ester **3.4***

In view of the problems associated with the synthesis of alcohol **3.5**, an alternative route to methyl ester **3.4** was required. One carbon chain homologation via the Arndt-Eistert procedure is known to be an efficient method for converting a carboxylic acid into the corresponding phenylacetic acid.[11,12] In general terms, the carboxylic acid is initially converted into the corresponding acid chloride, which is reacted with diazomethane to give the diazoketone. The diazoketone thereby formed can undergo a silver(I) promoted Wolff rearrangement to afford the desired phenylacetic acid.[13]

The application of Arndt-Eistert methodology should therefore provide access to phenylacetic acid **3.12** through the known carboxylic acid **3.13**. It is envisaged that the phenylacetic acid **3.12** could be readily converted into the corresponding methyl ester **3.4** through a simple esterification process (Scheme 3.7).

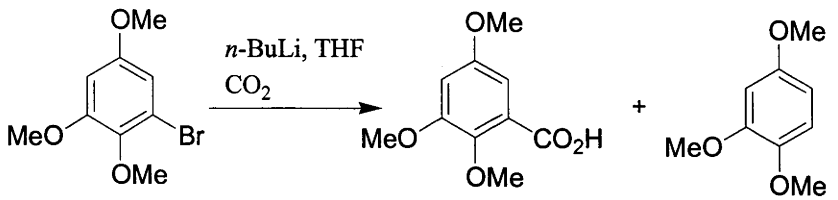


3.13 **3.12** **3.4**

*Scheme 3.7 Arndt-Eistert homologation approach to methyl ester **3.4***

The synthesis of carboxylic acid **3.13** from bromobenzene **3.6** has been achieved previously and initial attempts to access the required benzoic acid **3.13** followed the procedure developed by Kreuchunas for this conversion.[14,15] Accordingly, a THF solution of the lithio species of bromobenzene **3.6** was poured onto crushed solid carbon dioxide but only 1,2,4-trimethoxybenzene (**3.11**) was isolated from the reaction mixture.

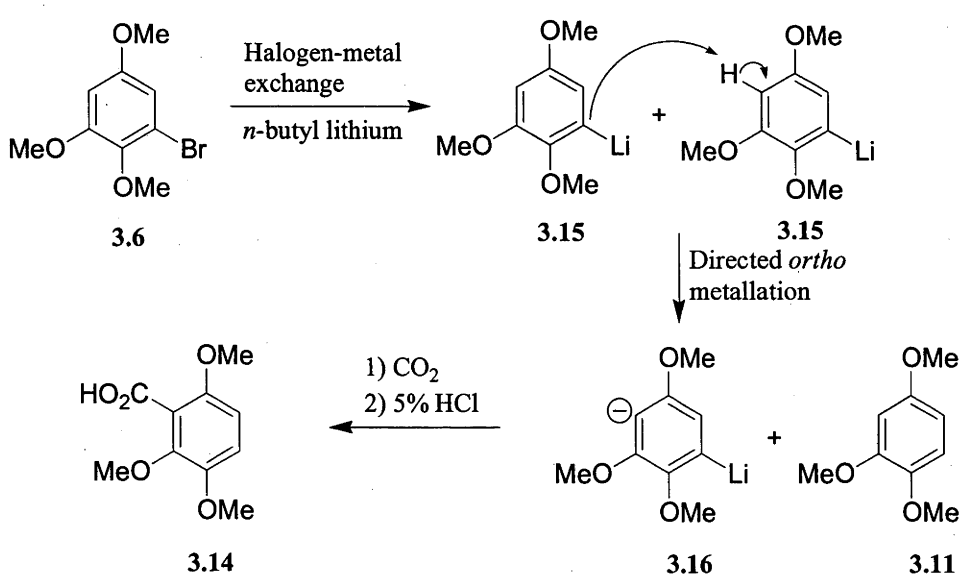
After much experimentation, the best yield of carboxylic acid **3.13** was obtained when bromobenzene **3.6** was treated with a slight excess of *n*-butyl lithium in THF, followed by the introduction of a steady stream of dry carbon dioxide gas and quenching of the reaction mixture with dilute acid (Scheme 3.8). The carboxylic acid **3.13** displayed two doublets at 7.12 ppm and 6.68 ppm in the proton NMR spectrum due to the two *meta*-coupled aromatic protons, along with three 3H signals corresponding to the methoxy protons.



3.6 **3.13** 92% **3.11** 8%

*Scheme 3.8 Synthesis of aromatic carboxylic acid **3.13***

When the concentration of bromobenzene **3.6** was increased threefold from 0.1M to 0.3M, the major product from the reaction was identified as 2,3,6-trimethoxybenzoic acid (**3.14**).[16] Presumably, halogen-metal exchange occurs to give the lithio species **3.15**, which, at this concentration, undergoes an intermolecular deprotonation under the *ortho* directing influence of the methoxy substituents to give the dianion **3.16** and 1,2,4-trimethoxybenzene (**3.11**) (Scheme 3.9). This dianion **3.16** is quenched with carbon dioxide and then water to give carboxylic acid **3.14**. The spectroscopic data for the carboxylic acid **3.14** thus isolated were identical to that reported in the literature.[16]

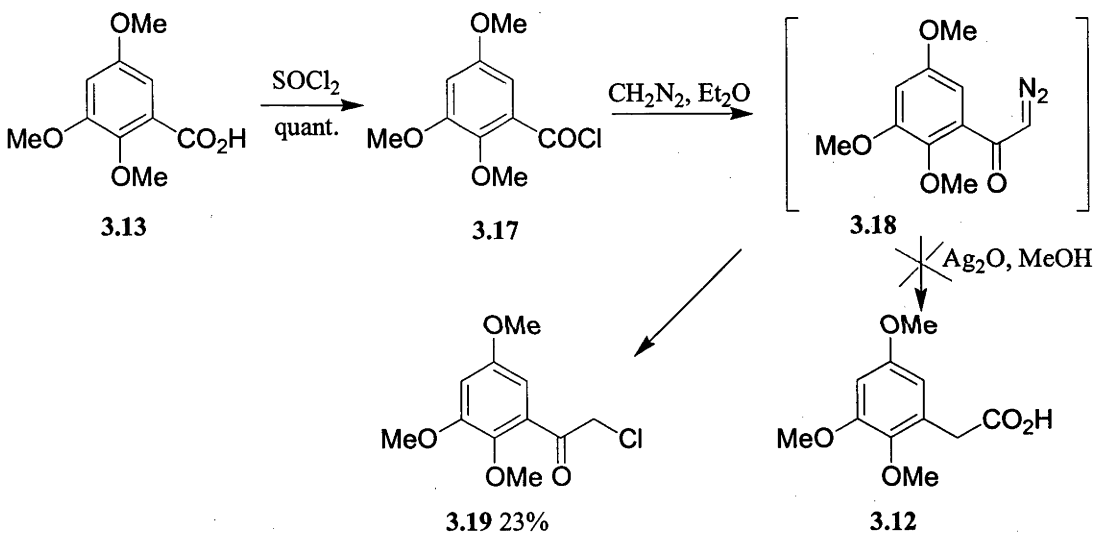


3.14 **3.15** **3.15** **3.16** **3.11**

*Scheme 3.9 Formation of carboxylic acid **3.14** at a high molar concentration of bromobenzene **3.6***

Following the successful synthesis of carboxylic acid **3.13**, the Arndt-Eistert homologation was attempted. The synthesis of acid chloride **3.17** was readily achieved by treatment of carboxylic acid **3.13** with thionyl chloride. The acid chloride was characterised by two doublets in the proton NMR spectrum at 6.69 ppm and 6.93 ppm, corresponding to the aromatic protons.

The acid chloride **3.17** was then treated with freshly prepared ethereal diazomethane, the solvent was removed *in vacuo* and the residue immediately added to a suspension of silver(I) oxide in boiling methanol. The reaction mixture was heated for two hours, during which time a further two aliquots of silver(I) oxide were added to the boiling solution. One major compound, ketone **3.19**, was isolated from the reaction mixture and purified via flash column chromatography (Scheme 3.10).



*Scheme 3.10 Attempted synthesis of phenylacetic acid **3.12***

The structure of **3.19** was elucidated by NMR spectroscopy and mass spectral analysis. The low-resolution electron impact mass spectrum displayed a molecular ion at *m/z* 244 with an isotopic pattern consistent with the presence of one chlorine atom in the molecule. High-resolution analysis of this ion indicated a molecular formula of $C_{11}H_{13}ClO_4$. The APT carbon NMR spectrum of the compound confirmed the presence of eleven distinct carbon environments. Three *O*-methyl carbons were evident at 55.58 ppm, 55.93 ppm and 61.46 ppm corresponding to the three methoxy substituents on the aromatic ring. Two distinct aromatic CH resonances and four quaternary aromatic carbon resonances were also evident, indicating that the substitution pattern on the

aromatic ring was analogous to the substitution pattern of the starting carboxylic acid **3.13**. A methylene carbon resonance was present at 50.43 ppm, consistent with the α-carbon of an α-halo ketone. One carbonyl carbon resonance was also present at 192.42 ppm, consistent with the carbonyl carbon of an α-halo ketone.

The long-range correlations observed in the gHMBC spectrum of the α-chloro ketone **3.19** confirmed this structural assignment (Figure 3.1). In particular, the $^{3}J_{CH}$ correlation between the aromatic proton at 6.70 ppm and the carbonyl carbon and the $^{2}J_{CH}$ correlation between the methylene protons at 4.64 ppm and the carbonyl carbon supported the assigned regiochemistry of the compound.

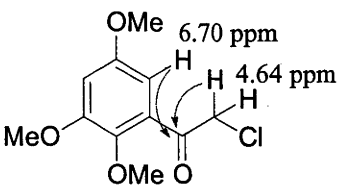
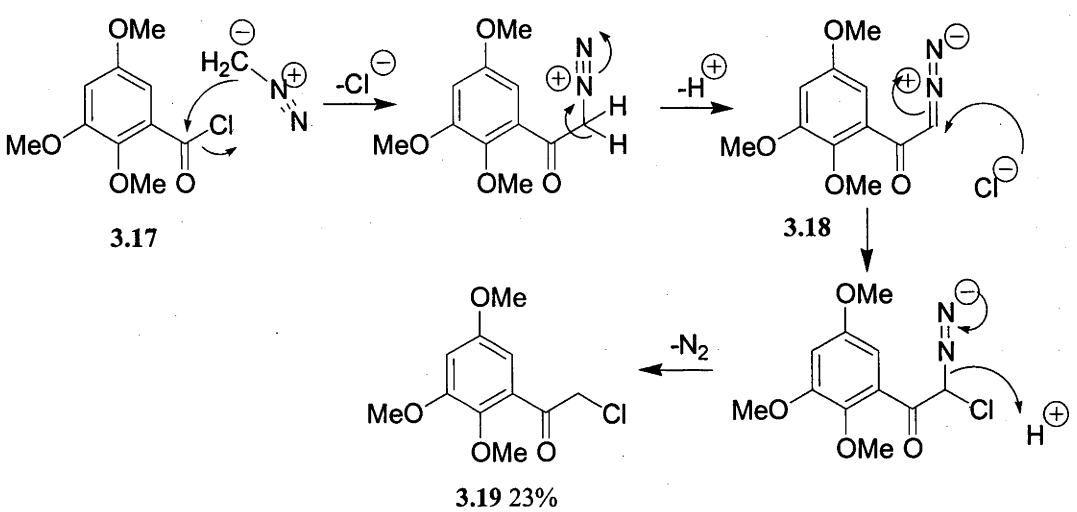


*Figure 3.1 Long range correlations observed in the gHMBC spectrum of ketone **3.19***

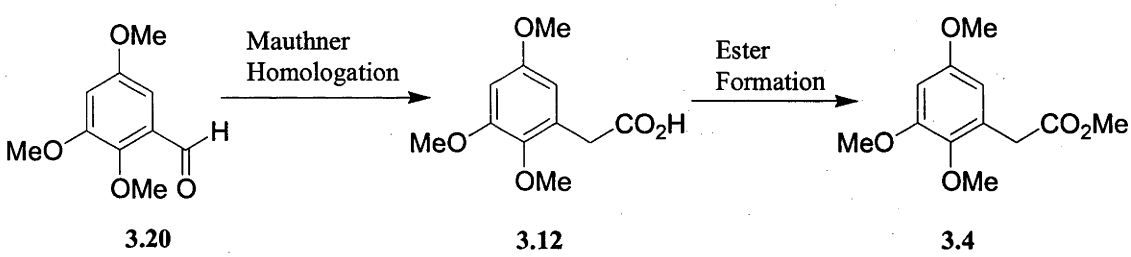
Newman and Beal have previously reported undesired side reactions of diazoketones with protic acids during their studies concerning the Wolff rearrangement.[17] The proposed mechanism for the formation of ketone **3.19** is shown in Scheme 3.11. The hydrochloric acid formed during the reaction of the acid chloride **3.17** with diazomethane presumably reacts with the diazoketone intermediate **3.18** at the α-carbon to give the by-product observed. The formation of this by-product suggests that the Wolff rearrangement of the diazoketone **3.18** does not occur under these reaction conditions.



*Scheme 3.11 Proposed mechanism for the formation of chloro derivative **3.19***

In view of the failure of the silver(I) oxide promoted Wolff rearrangement,[18] the use of silver(I) benzoate was examined but, unfortunately, only a complex mixture of products was obtained.[19,20] Due to the lack of success with the Arndt-Eistert approach to phenylacetic acid **3.12**, an alternative synthetic route to the methyl ester **3.4** was examined.

Smith and LaForge have reported the conversion of aldehyde **3.20** into the desired phenylacetic acid **3.12** via the Mauthner reaction.[21] Mauthner developed a general protocol for the conversion of aldehydes into aliphatic acids almost one hundred years ago.[22] Again, once access to phenylacetic acid **3.12** is achieved it should then be possible to synthesise the methyl ester **3.4** through a simple esterification reaction (Scheme 3.12).[23]



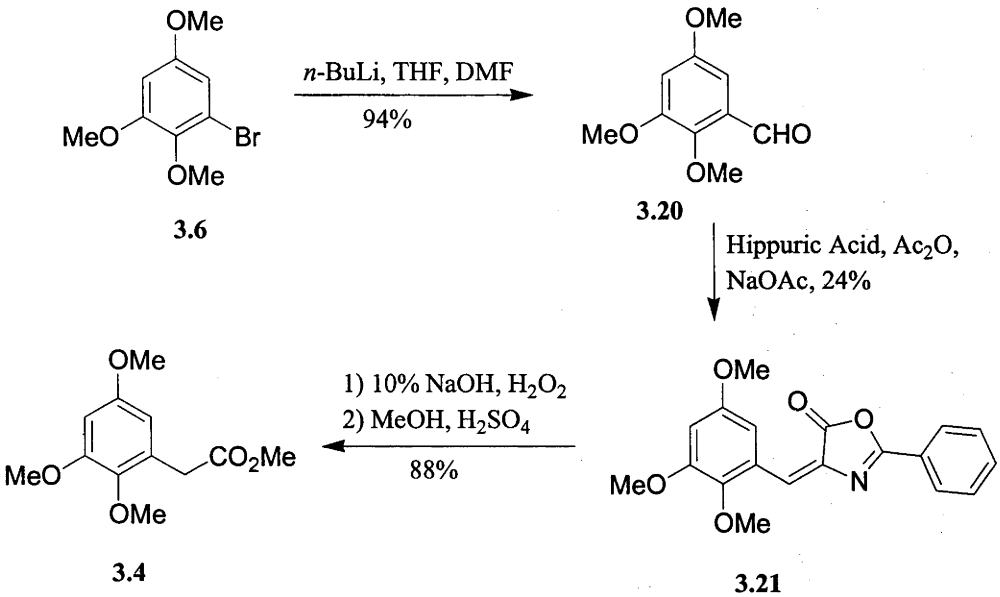
3.20 **3.12** **3.4**

*Scheme 3.12 Mauthner homologation approach to methyl ester **3.4***

The precursor aromatic aldehyde **3.20** has been synthesised by Merchant *et al.* in two steps, in poor overall yield, from commercially available *o*-vanillin.[24] Kessar and co-workers have also reported the synthesis of aldehyde **3.20** in their work towards the synthesis of naphthaphenanthridine alkaloids.[25] Experimental details were not published but they describe the synthesis as involving treatment of the Grignard reagent derived from bromobenzene **3.6** with *N*, *N*-dimethylformamide (DMF). In a similar manner, we treated bromobenzene **3.6** with *n*-butyl lithium and then quenched the reaction mixture with DMF to give the desired aldehyde **3.20** in 94% yield (Scheme 3.13).

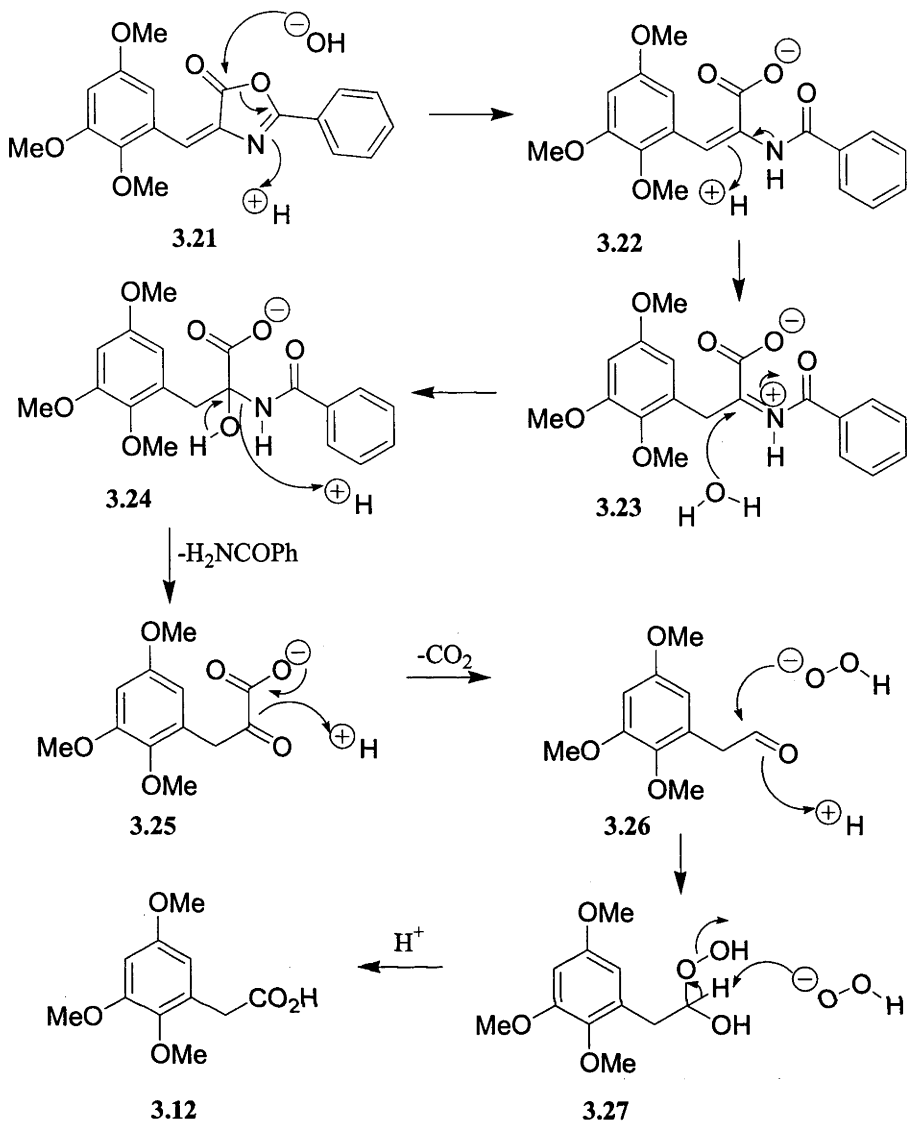
With the aldehyde **3.20** in hand, the methodology of Smith and LaForge was employed for the synthesis of phenylacetic acid **3.12**. Hence, the aldehyde **3.20** was reacted with hippuric acid in acetic anhydride containing anhydrous sodium acetate to give the intensely yellow azlactone **3.21** in low yield. The azlactone **3.21** was subsequently hydrolysed with aqueous 10% sodium hydroxide and then oxidised with hydrogen peroxide to give the corresponding phenylacetic acid **3.12**. The data obtained for the

phenylacetic acid **3.12** were consistent with that reported in the literature. The phenylacetic acid **3.12** was then converted to the methyl ester **3.4** in good yield through treatment with acidic methanol (Scheme 3.13).



*Scheme 3.13 Synthesis of methyl ester **3.4***

The mechanism for the conversion of the azlactone **3.21** to the phenylacetic acid **3.12** is thought to involve base catalysed hydrolysis to give the ring-opened azlactone derivative **3.22**, the protonation of which results in the formation of the zwitterionic derivative **3.23**. The zwitterion **3.23** is then hydrolysed to give the alcohol **3.24**, which fragments to form the ketone **3.25** and benzamide (Scheme 3.14). Loss of carbon dioxide from the ketone **3.25** occurs readily under basic conditions to afford the aldehyde **3.26**. The aldehyde **3.26** then reacts with the peroxide anion, generated *in situ* in the presence of sodium hydroxide, to afford the hydro peroxide **3.27**, which reacts with another peroxide anion to give rise to the desired phenylacetic acid **3.12** after acidic work up (Scheme 3.14).

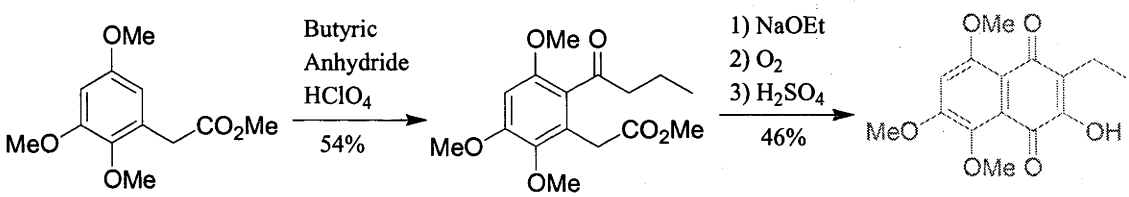


*Scheme 3.14 Proposed mechanism for phenylacetic acid **3.12** formation*

The methyl ester **3.4** was readily identified by the appearance of two doublets at 6.42 ppm and 6.30 ppm in the proton NMR spectrum corresponding to the two *meta*-coupled aromatic protons. A singlet at 3.64 ppm was also observed due to the benzylic methylene protons, along with four singlets corresponding to the four distinct methoxy group environments.

With the methyl ester **3.4** in hand, we then attempted a regioselective acylation using an excess of butyric anhydride in toluene and a complex mixture of products was obtained. When the reaction was performed in the absence of toluene, however, the methyl ester **3.4** was converted to the required ketone **3.3** in moderate yield (Scheme 3.15). The formation of this ketone **3.3** was evident by the appearance of a singlet at 6.41 ppm in the proton NMR spectrum due to the aromatic proton.

The ketone **3.3** was then treated with sodium ethoxide in ethanol to effect the Claisen condensation and subsequent aerial oxidation gave the 2-hydroxy-1,4-naphthoquinone **3.2** in moderate yield (Scheme 3.15). This bright orange solid was easily identified by the appearance of a characteristic quartet at 2.60 ppm and a triplet at 1.10 ppm in the proton NMR spectrum, attributable to the ethyl functionality on the quinonoid ring. Three methyl ether signals were also observed in the proton NMR spectrum, as were a singlet at 6.75 ppm and a broad singlet at 7.30 ppm due to the aromatic proton and the hydroxy group at the C2-position, respectively.



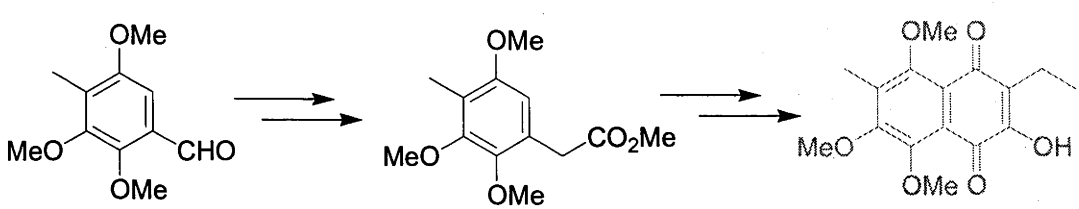
3.4 **3.3** **3.2**

*Scheme 3.15 Synthesis of 2-hydroxy-1,4-naphthoquinone **3.2***

These studies indicate, therefore, that the synthesis of 2-hydroxy-1,4-naphthoquinone **3.2** can be readily achieved from the methyl ester **3.4**, a derivative of phenylacetic acid **3.12**, which can be synthesised following literature procedures.

*3.3 The Synthesis of 2-Hydroxy-1,4-naphthoquinone **1.63***

An extension of these investigations to incorporate the synthesis of 2-hydroxy-1,4-naphthoquinone **1.63**, the quinone used by Nicolaou and Gray in their synthesis of hybocarpone (**1.25**)[1] was then desired. Access to naphthoquinone **1.63** should be possible from the appropriately functionalised methyl ester **3.28** which should, in turn, be accessible from the known aromatic aldehyde **3.29** (Scheme 3.16).[26]



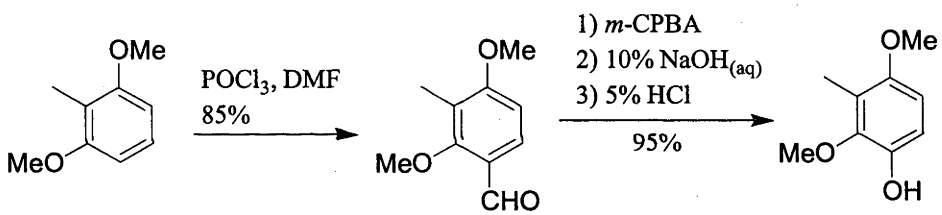
3.29 **3.28** **1.63**

*Scheme 3.16 Synthetic approach to 2-hydroxy-1,4-naphthoquinone **1.63***

3.3.1 The Synthesis of Aldehyde 3.29

Kitahara and co-workers have reported the synthesis of the required *C*-methyl benzaldehyde **3.29** as part of their studies towards the synthesis of indoloquinones.[27] The authors accessed the aldehyde **3.29** through a Duff formylation of phenol **3.30**. The synthesis of the precursor phenol **3.30** was achieved, by Godfrey *et al.*, in two steps from commercially available 2,6-dimethoxytoluene (**3.31**).[28] We therefore utilised these literature procedures to access the desired aldehyde **3.29**.

2,5-Dimethoxytoluene (**3.31**) was treated with phosphoryl chloride and DMF under Vilsmeier-Haack conditions to give the aromatic aldehyde **3.32**, the data for which were consistent with that reported in the literature.[28] The benzaldehyde **3.32** was then subjected to a Baeyer-Villiger oxidation using *m*-CPBA to afford the corresponding phenol **3.30**, after basic hydrolysis and acidic work-up (Scheme 3.17). The authors performed this oxidation reaction on 10mmol of precursor aldehyde **3.32**. In our hands, difficulties were experienced when the reaction was attempted on a larger scale.



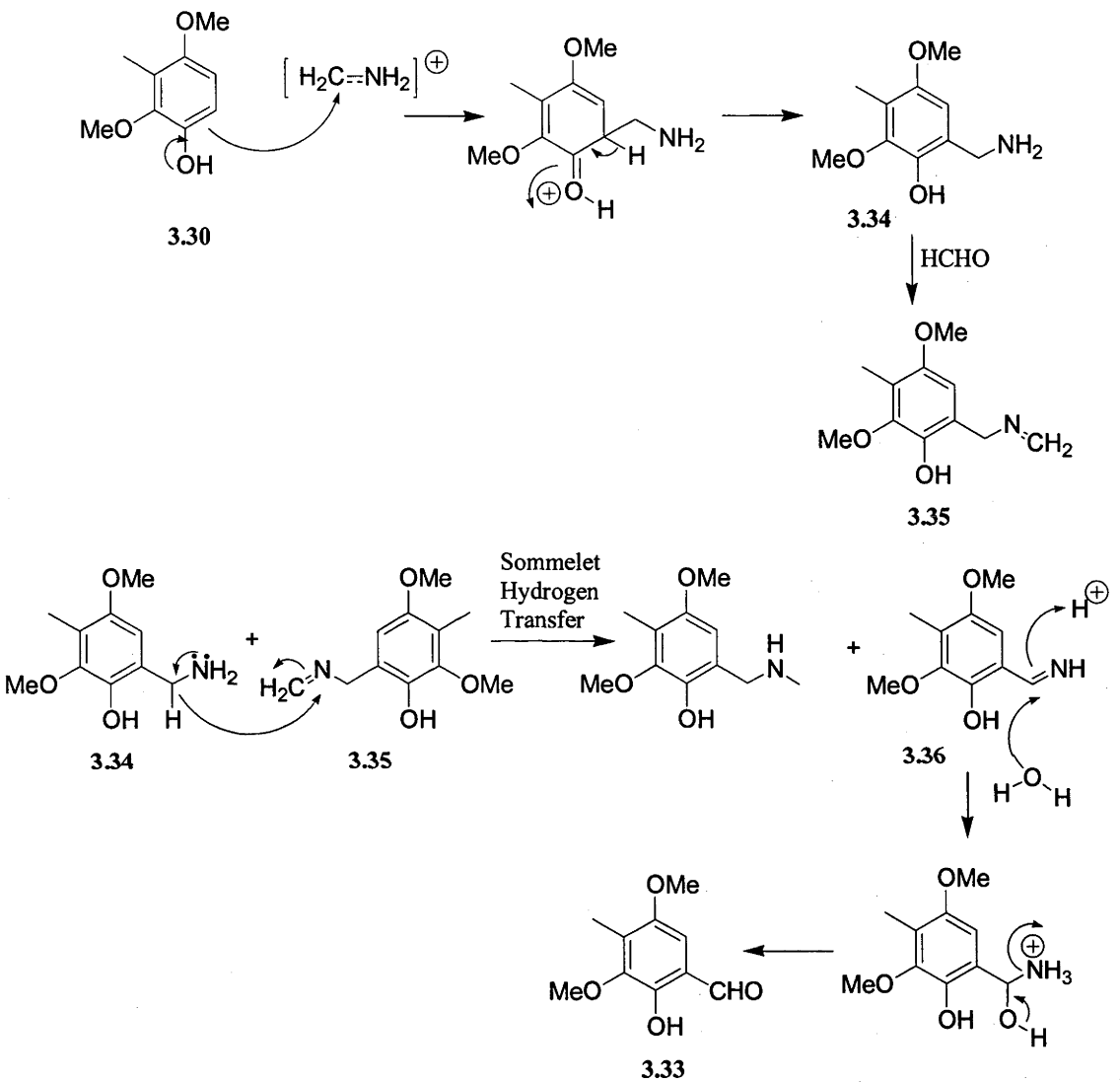
3.31 **3.32** **3.30**

Scheme 3.17 Synthesis of phenol 3.30

The Duff reaction, using hexamethylenetetraamine (hexamine) as a source of electrophilic carbon, was then employed to convert phenol **3.30** into aldehyde **3.33**, following the methodology developed by Kitahara and co-workers. The aldehyde **3.33** was isolated in good yield and was characterised by a singlet at 9.76 ppm due to the aldehydic proton, and a singlet at 6.65 ppm due to the aromatic proton, in the proton NMR spectrum.

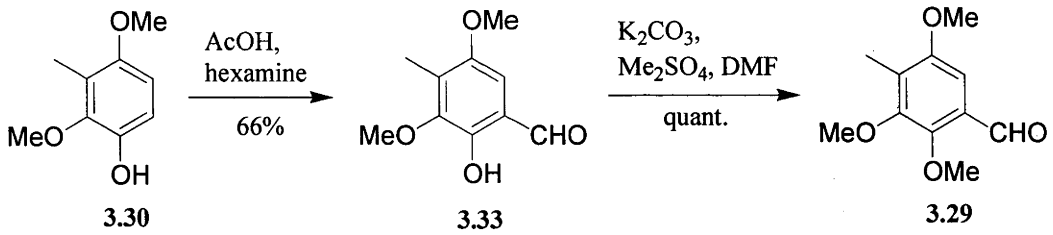
The Duff formylation selectively installs an aldehyde functionality *ortho* to a hydroxy group in an aromatic system. Ogata and co-workers have examined the kinetics and mechanism of the Duff reaction in detail for naphthalene systems.[29] Applying their mechanistic conclusions to this reaction, the mechanism involves *ortho* directed attack of the phenol **3.30** onto the electrophilic hexamine fragment. The resultant amine **3.34**

then reacts with formaldehyde liberated under the reaction conditions to give imine **3.35**. A Sommelet hydrogen transfer between the imine **3.35** thus formed and amine **3.34** then gives rise to imine **3.36**, which is hydrolysed under the aqueous work-up conditions to give the aldehyde **3.33** (Scheme 3.18).[30]



Scheme 3.18 Ogata et al. *mechanism of the Duff reaction*

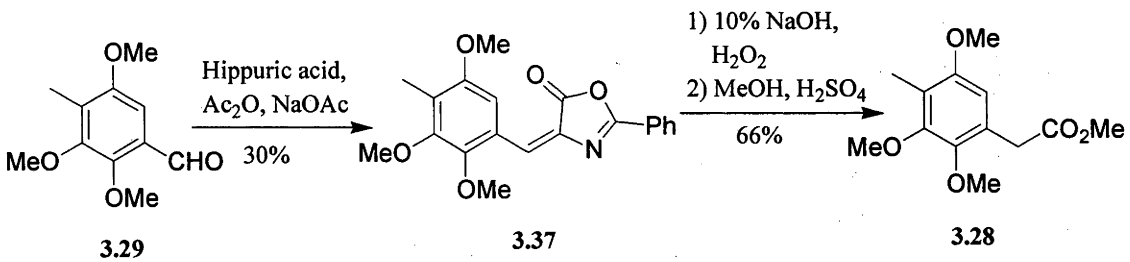
The aldehyde **3.33** was then converted into the desired 2,3,5-trimethoxy-4-methylbenzaldehyde (**3.29**) under *O*-alkylation conditions. The spectroscopic data for aldehyde **3.29** thus synthesised were identical to that reported in the literature (Scheme 3.19).[27]



*Scheme 3.19 Synthesis of aldehyde **3.29***

*3.3.2 The Synthesis of Methyl Ester **3.28** via Azlactone Route*

With the appropriate aromatic aldehyde **3.29** in hand, we then attempted the synthesis of methyl ester **3.28** through the application of the methodology outlined in Scheme 3.14 for the synthesis of methyl ester **3.4**. The aldehyde **3.29** was reacted with hippuric acid under acidic conditions to give the corresponding azlactone **3.37** in poor yield. The azlactone **3.37** was identified by the appearance of a singlet at 7.69 ppm in the proton NMR spectrum due to the vinylic proton at the benzylic position. Hydrolysis and oxidation of the azlactone **3.37** then gave the corresponding phenylacetic acid, which was converted into the desired methyl ester **3.28** in excellent yield through treatment with acidic methanol (Scheme 3.20). Unfortunately, the yields for the azlactone **3.37** rearrangement reaction were inconsistent and by-product formation was frequently evident.



*Scheme 3.20 The synthesis of methyl ester **3.28** via the azlactone **3.37***

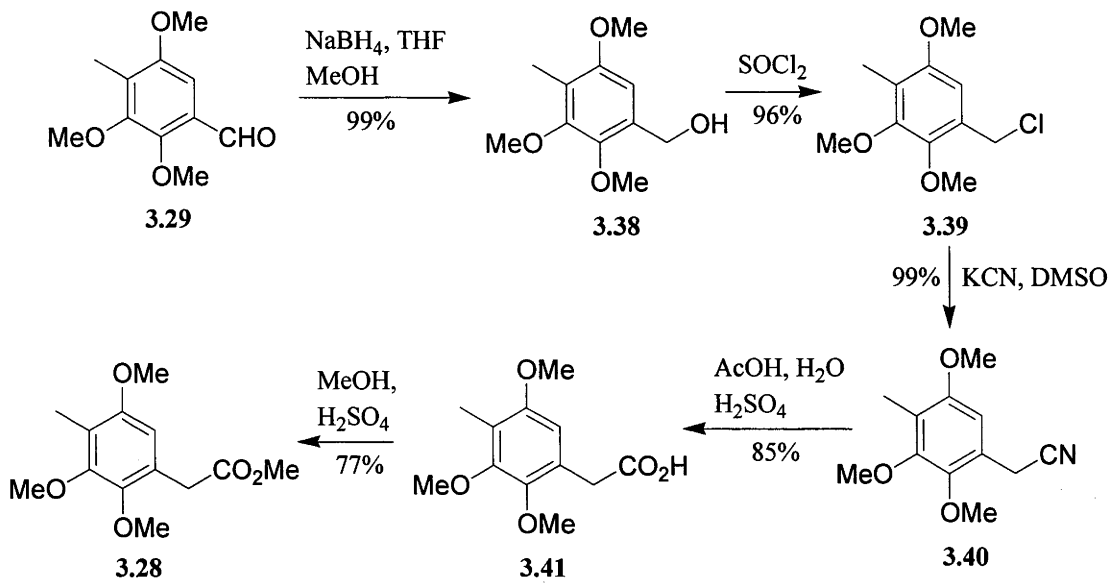
*3.3.3 The Synthesis of Methyl Ester **3.28** via the Cyano Route*

As we were unable to isolate satisfactory quantities of the methyl ester **3.28** from azlactone **3.37**, an alternative means of accessing the ester was examined. There are many reports in the literature concerning the conversion of an aldehyde into the corresponding aliphatic acid via a one-carbon chain homologation using cyanide as the source of the carbon atom.[31] In general terms, the aldehyde is reduced to the

corresponding alcohol and then the alcohol functionality is converted into a better leaving group. The displacement of this leaving group with the cyanide ion yields the cyano derivative, which is hydrolysed to reveal the chain extended aliphatic acid.

In order to examine this approach, it was therefore necessary to reduce the benzaldehyde **3.29** to give the corresponding benzyl alcohol **3.38**. Hence, the aromatic aldehyde **3.29** was initially reacted with 0.25 equivalents of lithium aluminium hydride. A number of undesired by-products were evident, along with starting material, in the proton NMR spectrum and so an alternative reducing agent was sought. When sodium borohydride was used as the reducing agent, the desired benzyl alcohol **3.38** was formed in excellent yield (Scheme 3.21). The benzyl alcohol **3.38** was characterised by a singlet at 4.64 ppm in the proton NMR spectrum due to the methylene protons.

The benzyl alcohol **3.38** was then converted into the benzyl chloride **3.39** through treatment with thionyl chloride. The benzyl chloride **3.39** was characterised by a diagnostic resonance at 41.5 ppm in the carbon NMR spectrum due to the benzylic carbon. The benzyl chloride **3.39** was then reacted with potassium cyanide to give the cyano derivative **3.40**, through a nucleophilic substitution reaction. The cyano derivative **3.40** was isolated following an aqueous work-up and displayed a diagnostic methylene signal at 3.68 ppm in the proton NMR spectrum. This significant upfield shift of the benzylic protons when compared to the benzyl chloride **3.39** and benzyl alcohol **3.38** is due to the proximity of the protons to the shielding carbon-nitrogen triple bond. The cyano derivative **3.40** was then hydrolysed under acidic conditions to give the corresponding phenylacetic acid **3.41** in excellent yield. The phenylacetic acid **3.41** was then converted into the required methyl ester **3.28** using acidic methanol (Scheme 3.21).

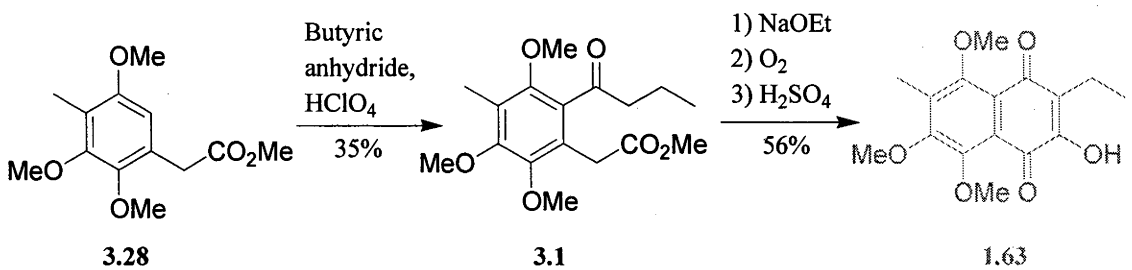


*Scheme 3.21 Cyanide homologation approach to the synthesis of methyl ester **3.28***

*3.3.4 The Synthesis of 2-Hydroxy-1,4-naphthoquinone **1.63***

With the desired methyl ester **3.28** in hand, the synthesis of 2-hydroxy-1,4-naphthoquinone **1.63** was then attempted. The successful acylation of methyl ester **3.28** was achieved using butyric anhydride and ketone **3.1** was accessed in moderate yield (Scheme 3.22). The formation of ketone **3.1** was evident by the disappearance of the aromatic proton signal at 6.45 ppm in the proton NMR spectrum attributable to the starting material, methyl ester **3.28**.

A Claisen condensation reaction was then performed to give the desired 2-hydroxy-1,4-naphthoquinone **1.63** after aerial oxidation and acidic work-up. The spectroscopic data for 2-hydroxy-1,4-naphthoquinone **1.63** were identical to that reported by Nicolaou and Gray and this therefore constitutes a formal, selective total synthesis of hybocarpone (**1.25**).



*Scheme 3.22 Synthesis of 2-hydroxy-1,4-naphthoquinone **1.63***

3.4 Conclusion

We have successfully synthesised two 2-hydroxy-1,4-naphthoquinones, including the synthetic precursor to hybocarpone (**1.25**), through the application of the Claisen condensation/oxidation protocol to the appropriately substituted ketones **3.1** and **3.3**. This is a facile and potentially versatile approach to the synthesis of highly functionalised naphthoquinones.

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Chapter Four:
Towards the Synthesis of
Bis-*Naphthazarin Derivatives*

4.1 Introduction

As discussed previously, dimeric naphthoquinones have been isolated from a variety of natural sources and are of interest due to the bioactivity displayed by several lead compounds (Chapter One). These natural products differ significantly in the nature of the dimeric linkage, which can involve carbon-carbon or carbon-oxygen bonds and, as such, they can be described as C-C or C-O dimers respectively. For example, the lichen metabolite, islandoquinone (**1.24**), is a C-O dimer isolated recently in Russia (Figure 4.1).[1]

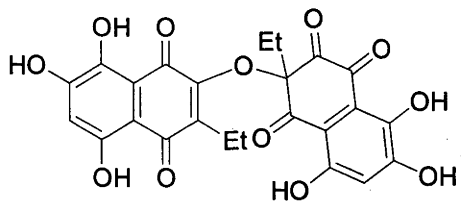
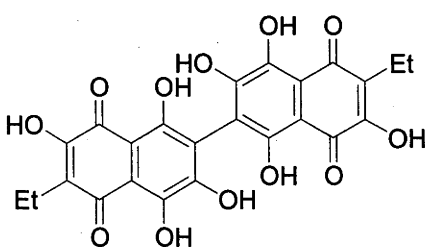
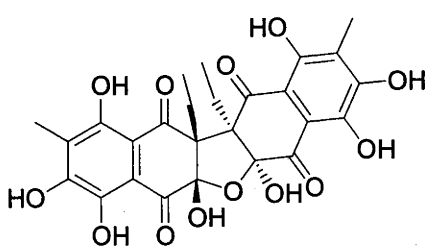


*Figure 4.1 Islandoquinone (**1.24**)*

Naphthoquinones consist of fused quinonoid and arene ring systems and so the dimeric linkage can be further described as being quinonoid-quinonoid, quinonoid-arene or arene-arene in nature. For example, the marine natural product 6,6'-*bis*-(3-ethyl-2,7-dihydroxynaphthazarin) (**1.20**) is a C-C dimer containing an arene-arene linkage (Figure 4.2).[2] Another structural feature concerns the number and type of bonds involved in the linkage, as the naphthoquinonoid moieties can be joined through a single bond, as in islandoquinone (**1.24**) and the *bis*-naphthazarin **1.20**, an alkyl bridge or a fused ring system. An example of the latter is hybocarpone (**1.25**), which contains a fused furanoid hemiacetal linkage between the naphthoquinonoid moieties, a structural feature that has not been previously observed in natural products.[3]



1.20

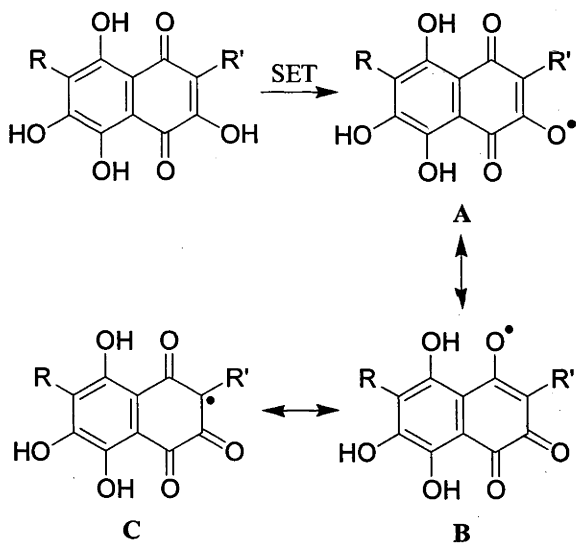


1.25

Figure 4.2 Bis-*naphthazarin **1.20** and hybocarpone (**1.25**)*

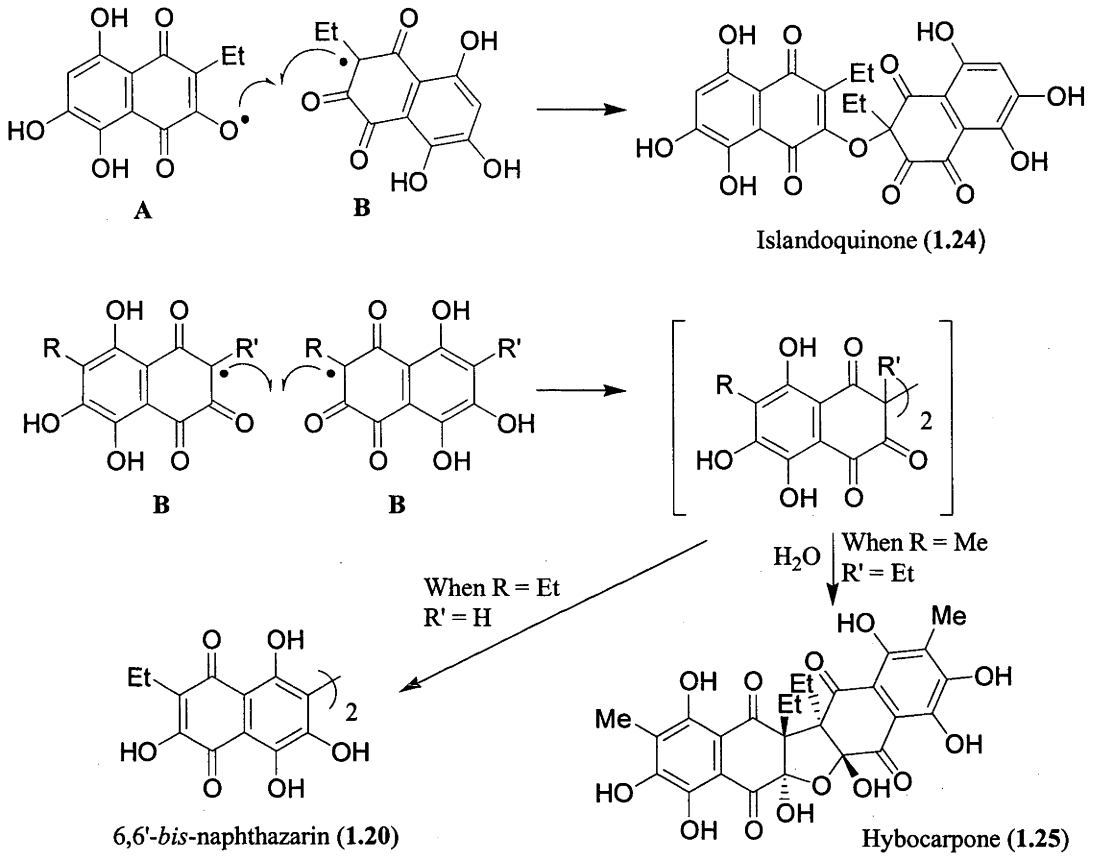
4.1.1 Postulated Biosynthesis of Bis-*Naphthazarin Derivatives*

These natural products exemplify the structural diversity of *bis*-naphthazarin derivatives witnessed in Nature. The synthetic challenge that compounds of this type pose is therefore significant and an understanding of their biosynthesis may illuminate some key issues in the dimerisation process. The biosynthesis of *bis*-naphthazarin derivatives is thought to involve the coupling of two monomeric subunits. A single electron transfer (SET) reaction may generate an *O*-centred radical species such as A, which can be represented by other resonance structures such as B and C (Scheme 4.1). Enzyme mediated coupling of two radical centres derived from the monomeric naphthazarin can therefore lead to different intermediate dimers which can undergo further reactions to give rise to *bis*-naphthazarin derivatives.



Scheme 4.1 Postulated radical generation via single electrontransfer (SET)

For example, carbon-oxygen radical coupling could result in C-O dimers such as islandoquinone (**1.24**), whereas carbon-carbon radical coupling presumably leads to C-C type dimers such as the *bis*-naphthazarin **1.20** and hybocarpone (**1.25**), when, in the latter case, this is followed by a water-mediated furanoid ring closure (Scheme 4.2).

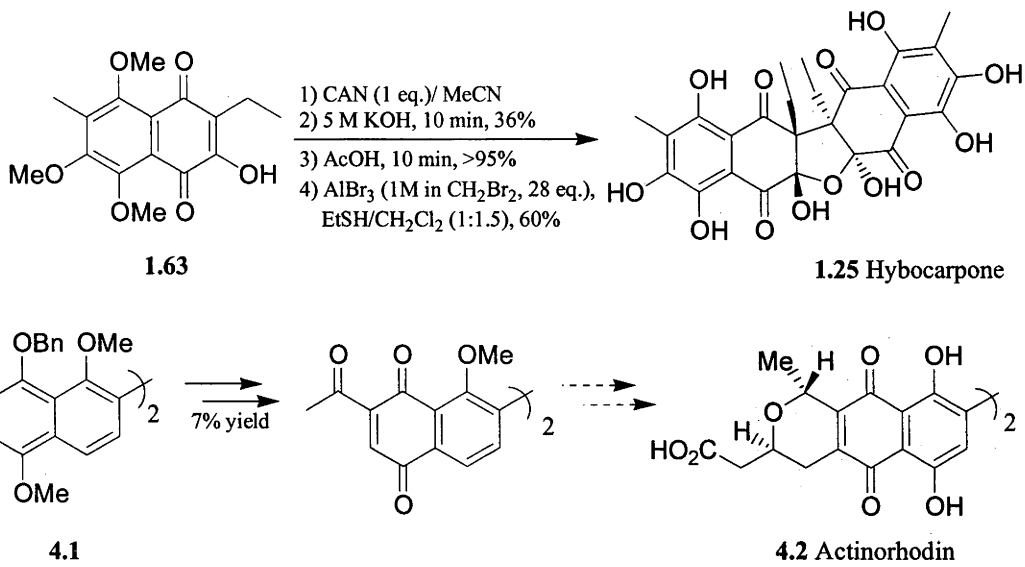


Scheme 4.2 Proposed biosynthesis of naturdly occurring bis-*naphthazarin derivatives*

It is therefore evident that, although a large number of dimers are possible via this pathway, enzymatic control dictates the formation of particular products by a given organism. The biosynthesis of hybocarpone (**1.25**) involves the generation of four contiguous stereogenic centres and, as a result, there are potentially sixteen (2^4) stereoisomeric outcomes. Hybocarpone (**1.25**) was, however, isolated as a single stereoisomer, suggesting that the dimerisation process is enzyme mediated and stereocontrolled.

4.1.2 Known Syntheses of Bis-Naphthazarin Derivatives

While Nature has successfully synthesised a range of structurally complex dimers, there are very few known chemical syntheses of *bis*-naphthazarins.[4] In general, synthetic approaches to *bis*-naphthazarins have involved the dimerisation of *O*-alkylated precursors such as **1.63** and **4.1** prior to deprotection to reveal the *bis*-naphthazarin, as in Nicolaou's synthesis of hybocarpone (**1.25**)[5] and Brimble's approach to actinorhodin (**4.2**)[6] (Scheme 4.3).

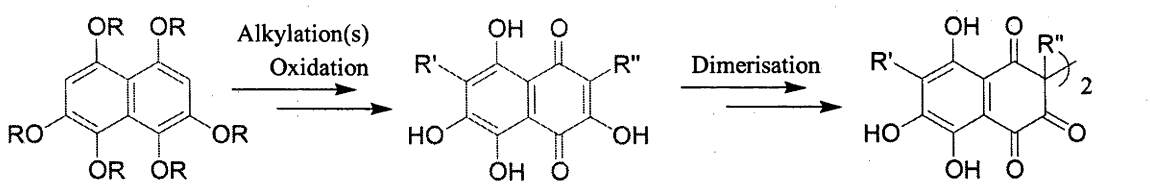


1.63 **1.25** Hybocarpone

4.1 **4.2** Actinorhodin

Scheme 4.3 Synthetic approaches to bis-*naphthazarin derivatives*

While this approach has been moderately successful, we wanted to examine the dimerisation of naphthazarins, in order to provide a biomimetic route to such compounds. Although it would be difficult to predict the regiochemical and stereoisomeric nature of dimers formed via this approach, useful insights into the mechanisms of dimerisation may be gained. Thus, with several naphthazarins in hand (Chapter Two), an investigation into the dimerisation of the monomeric naphthazarins was initiated (Scheme 4.4).



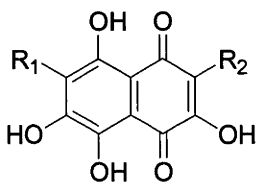
Scheme 4.4 Our synthetic approach to bis-*naphthazarins*

4.2 Towards the Synthesis of Bis-*Naphthazarin Derivatives*

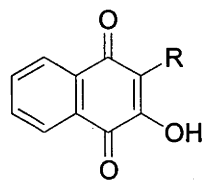
The synthesis of dimeric naphthoquinones has been evaluated extensively[7-12] and so our investigations were initially focused on utilising the conditions and reagents known to effect the dimerisation of naphthoquinones.

4.2.1 The Naphthazarins and Naphthoquinones under Investigation

The naphthazarins boryquinone (**1.16**), aureoquinone (**1.14**) and diethylmompain (**2.53**) were utilized during the course of this investigation (Chapter Two). As only limited quantities of the synthetically derived naphthazarins were available, and to further our general understanding of the dimerisation of naphthoquinones, the behaviour of the naturally occurring 1,4-naphthoquinones, lawsone (**1.3**) and phthiocol (**1.7**), was also examined (Figure 4.3).



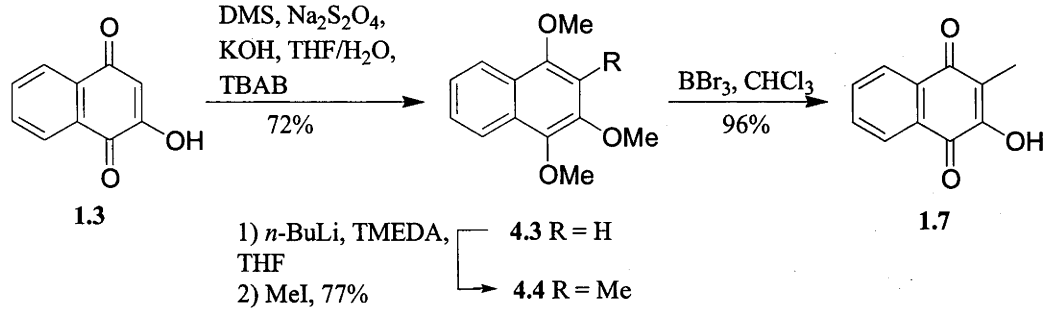
1.16 R_1 = Me, R_2 = Et: Boryquinone
1.14 R_1 = R_2 = Me: Aureoquinone
2.53 R_1 = R_2 = Et: Diethylmompain



1.3 R = H: Lawsone
1.7 R = Me: Phthiocol

Figure 4.3 Naphthazarins and naphthoquinones under investigation

The synthesis of phthiocol (**1.7**) from 2-methyl-1,4-naphthoquinone (menadione, **1.2**) has been reported by Fieser in 1940[13] and by Srivastava *et al.* in 1987 using the same methodology.[14] However, given the large quantities of lawsone (**1.3**) available, we developed an alternative synthetic route starting from this naphthoquinone. Thus, the reductive alkylation of lawsone (**1.3**) gave the *O*-methyl protected naphthalene **4.3** in very good yield. The resultant 1,2,4-trimethoxynaphthalene (**4.3**) was then regioselectively *C*-alkylated to give naphthalene **4.4** through treatment with *n*-butyl lithium followed by the quenching of the lithio species thus generated with methyl iodide. The desired naphthoquinone **1.7** was then accessed in excellent yield via cleavage of the aryl methyl ethers with an excess of boron tribromide (Scheme 4.5).

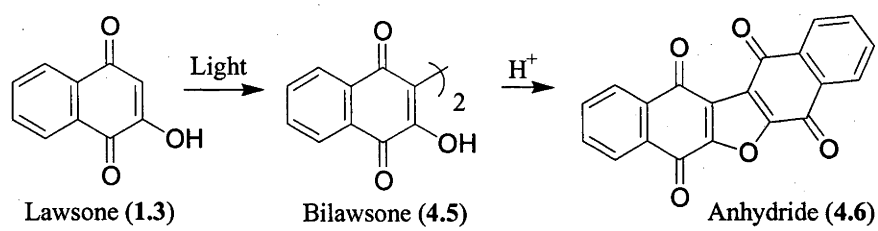


Scheme 4.5 Synthesis of phthiocol (1.7)

This synthetic route is potentially more versatile than that previously reported as, in principle, a number of different alkyl groups can be installed at the C3-position through treatment of the lithio species generated from naphthalene **4.3** with the appropriate alkyl halides.

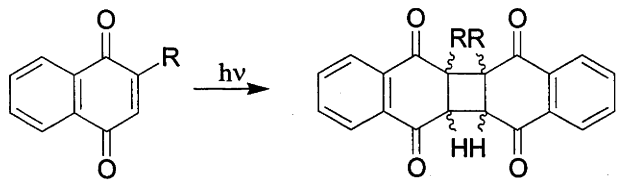
4.2.2 Known Photolytic Dimerisations of Naphthoquinones

The *in situ* generation of radical species under photolytic conditions has been successfully employed in the synthesis of naphthoquinone dimers. Hooker observed that lawsone (**1.3**) was converted to the C-C dimer, bilawsone (**4.5**), when an aqueous solution was exposed to sunlight. This result could be replicated when a Uviarc lamp was used (Scheme 4.6). The bilawsone (**4.5**) was subsequently converted to the fused ring anhydride (**4.6**) through treatment with aqueous acid, thereby altering the nature of the dimeric linkage.[15] In 1994, Suginome *et al.* re-examined the photochemical behaviour of lawsone (**1.3**) and synthesised bilawsone (**4.5**) in 45% yield using a 500 W high-pressure mercury lamp.[16]



Scheme 4.6 Dimerisation of lawsone (1.3) and formation of anhydride 4.6

However, the photochemical behaviour of naphthoquinones does not always result in the synthesis of simple C-C dimers. Werbin and Strom investigated the effect of near-ultraviolet radiation and sunlight on menadione (**1.2**) and isolated several stereoisomeric cyclobutane photodimers.[17] Taira and co-workers re-investigated this reaction and confirmed the structures of the photodimers via X-ray crystallography.[18] Similar photodimers have also been isolated following the irradiation of 1,4-naphthoquinone (**1.1**)[19,20] and *O*-methyl lawsone (**4.7**)[21] (Scheme 4.7). A [2+2] cycloaddition reaction between two naphthoquinones presumably gives rise to these cyclobutane derivatives.



1.2 R = Me
1.1 R = H
4.7 R = OMe

Scheme 4.7 Photodimerisation of naphthoquinones

These studies suggest that the formation of a simple C-C dimer, such as bilawsone (**4.5**), under photolytic conditions requires the hydroxy functionality at the C2-position and may involve radical precursors such as those described in the postulated biosynthetic pathway to *bis*-naphthazarins (Scheme 4.2).

4.2.3 Photolytic and Electrolytic Investigations

Given the conceptual simplicity of the photolytic dimerisation of naphthoquinones, as well as the fact that the naphthazarins under investigation all contain the C2-hydroxy functionality, our initial studies into the dimerisation of naphthoquinones and naphthazarins involved these methods.

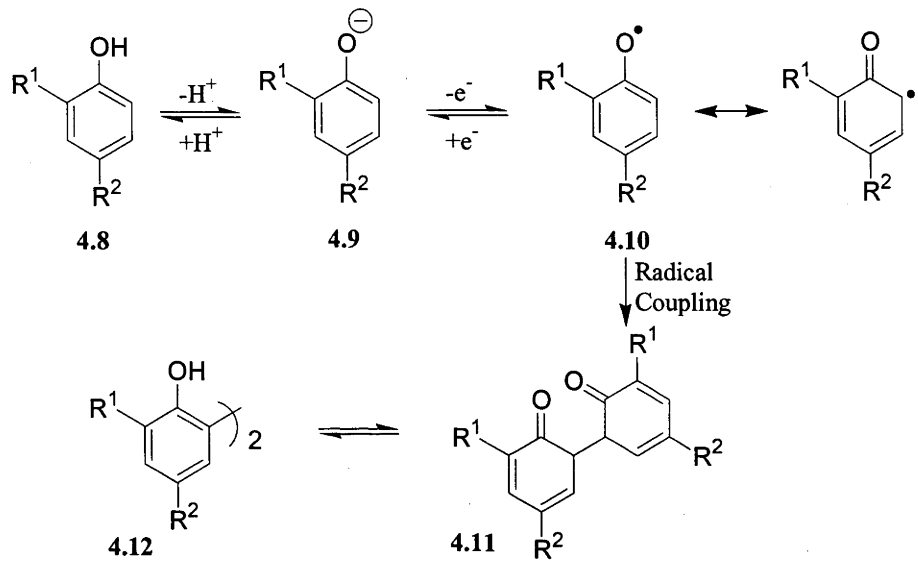
Lawsone (**1.3**) was dissolved in a minimal amount of water and irradiated with a 100 W tungsten lamp at 80°C. The reaction was monitored by reverse-phase HPLC and the synthesis of bilawsone (**4.5**) was confirmed through ultraviolet/visible spectral comparison with the authentic material at 254 nm. The reaction proceeded with 80% conversion of lawsone (**1.3**) to bilawsone (**4.5**) after six hours. When aureoquinone (**1.14**) and

boryquinone (**1.16**) were treated under analogous conditions, however, no reaction was observed after 24 hours and the starting materials were recovered.

Given that sunlight could be used to convert lawsone (**1.3**) into bilawsone (**4.5**), and that the exact wavelength of light required for the dimerisation is unknown, we investigated the use of natural light. As the quinones display only limited water solubility, methanolic solutions of lawsone (**1.3**), phthiocol (**1.7**) and aureoquinone (**1.14**) were exposed to direct sunlight and the solutions were analysed by HPLC after various time intervals. Complete conversion of lawsone (**1.3**) to bilawsone (**4.5**) was observed after three months. The methanolic solution of phthiocol (**1.7**), however, contained only starting material and aureoquinone (**1.14**) decomposed to give a complex mixture of products over the same period of time.

As photolytic conditions failed to yield dimers of phthiocol (**1.7**), aureoquinone (**1.14**) or boryquinone (**1.16**), an alternative synthetic approach was sought. The oxidative coupling of a wide variety of substrates, including phenols, naphthalenes, conjugated dienes and carboxylic acids has been achieved under electrolytic conditions.[22] Dimeric 1,4-naphthoquinones have been synthesised previously in this manner by du Plessis and co-workers,[23] and so electrosynthesis could provide an alternative avenue for dimerisation studies.

In general terms, electrosynthesis involves the application of a voltage greater than the oxidation potential of the substrate. This process has been studied extensively for oxidative phenolic coupling and the accepted mechanism for this process is outlined in Scheme 4.8. The phenolic substrate **4.8** dissociates to the anion **4.9** and the applied voltage then results in the generation of radical species **4.10** through the loss of one electron. Radical coupling of species **4.10** ensues to give the dimeric derivative **4.11** which can tautomerise to give the biaryl derivative **4.12** (Scheme 4.8).

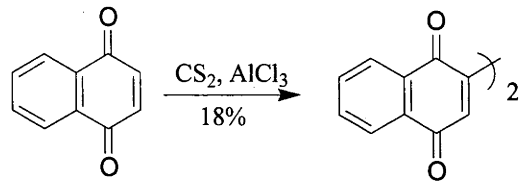


Scheme 4.8 Electrolytic oxidative phenolic coupling

The electrochemical profiles of lawsone (**1.3**) and phthiocol (**1.7**) have been measured under various conditions.[24-27] Our attempts at the electrosynthesis of bilawsone (**4.5**) initially involved an investigation into the behaviour of lawsone (**1.3**) in acetonitrile across a large potential range. A 2.2V current was then passed through the cell for 18 hours, following which the solution was analysed by HPLC. From this a 9% conversion of lawsone (**1.3**) to bilawsone (**4.5**) was evident. Although the conditions required for electrosynthesis of bilawsone (**4.5**) were not optimised but boryquinone (**1.16**) was treated analogously, following an analysis of its electrochemical profile. A complex mixture of products was evident from HPLC analysis and so this approach was abandoned.

4.2.4 Naphthoquinone Dimerisation under Oxidative Conditions

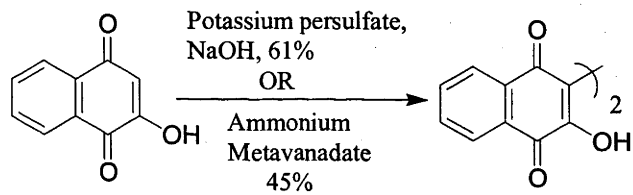
The dimerisation of naphthoquinones has also been achieved using a range of chemical oxidants. For example, Buchan and Musgrove reported the reaction of 1,4-naphthoquinone (**1.1**) with carbon disulfide and anhydrous aluminium trichloride to give the corresponding C-C dimer **4.13** in just 18% yield (Scheme 4.9).[28]



1.1 **4.13**

Scheme 4.9 Dimerisation of 1,4-naphthoquinone (1.1)

The dimerisation of lawsone (**1.3**) using chemical oxidants has been reported by a number of authors to proceed with moderate yields. Chandrasenan and Thomson[29] reported the use of aqueous potassium persulfate and sodium hydroxide, while Hazra *et al.*[30] employed ammonium metavanadate for the dimerisation of lawsone (**1.3**) to bilawsone (**4.5**) (Scheme 4.10).



1.3 **4.5**

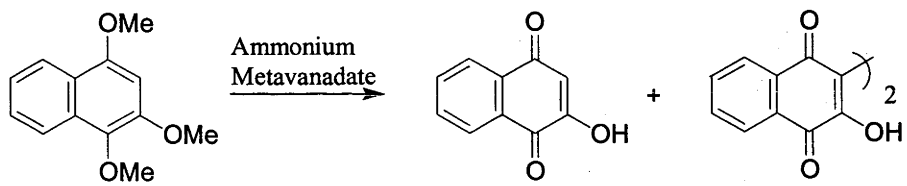
Scheme 4.10 Dimerisation of lawsone (1.3)

As persulfate salts have also been used in the synthesis of several other naphthoquinone dimers,[31,32] we investigated the use of this oxidant. When lawsone (**1.3**) was treated under the basic conditions of Chandresanan and Thomson, bilawsone (**4.5**) was isolated in 80% yield following purification. The formation of by-products, however, was evident from HPLC analysis of the crude product mixture. When diethylmompain (**2.53**) and phthiocol (**1.7**) were treated with potassium persulfate under these basic conditions, complex mixtures of products were obtained.

Forrester *et al.* have successfully utilised persulfate salts in the absence of sodium hydroxide for the dimerisation of base sensitive aminoquinones.[33] When lawsone (**1.3**) was treated with persulfate under neutral conditions, we obtained bilawsone (**4.5**) in quantitative yield. However, when diethylmompain (**2.53**) was treated under these conditions only

starting material was recovered, whereas the analogous reaction of phthiocol (**1.7**) led to a complex mixture of products.

As ammonium metavanadate has been employed for the successful synthesis of bilawsone (**4.5**), the dimerisation of aureoquinone (**1.14**) was also attempted using this reagent. Disappointingly, a complex mixture of products was obtained. Hazra *et al.* also reported the use of ammonium metavanadate for the dimerisation of naphthalenes.[30] In a similar manner, we examined the reaction of the naphthalene **4.3** (Scheme 4.5) with this reagent and found that when 1,2,4-trimethoxynaphthalene (**4.3**) was treated with ammonium metavanadate overnight, HPLC analysis indicated that the product mixture comprised a 1:4 mixture of bilawsone (**4.5**) and lawsone (**1.3**) (Scheme 4.11).



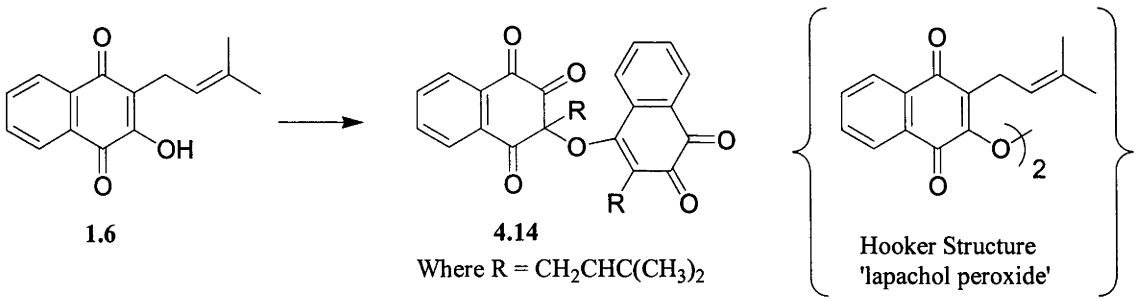
4.3 **1.3** **4.5**

Scheme 4.11 Oxidative demethylation and dimerisation of trimethoxynaphthalene ***4.3***

The ability of ammonium metavanadate to effect aryl ether cleavage as well as oxidative dimerisation was interesting as there are few reagents known to effect such an oxidative deprotection cleanly.

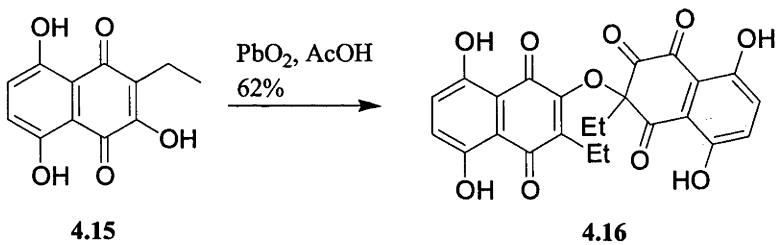
4.2.5 Studies involving Lead(IV) Oxide

From the studies described above, it was evident that the dimerisation of lawsone (**1.3**) could be readily achieved under a variety of conditions. The dimerisations of 3-substituted-2-hydroxy-1,4-naphthazarins, however, are clearly problematic. In 1936, Hooker reported the synthesis of 'lapachol peroxide', a dimer formed from the 3-alkyl substituted 2-hydroxy-1,4-naphthoquinone, lapachol (**1.6**), through treatment with lead(IV) oxide in glacial acetic acid.[34] The structure proposed was that of an organic peroxide, with an O-O dimeric linkage. When this reaction was re-examined by Ettlinger, the C-O dimeric structure **4.14** was assigned to the product through analysis of the UV spectrum (Scheme 4.12).[35]



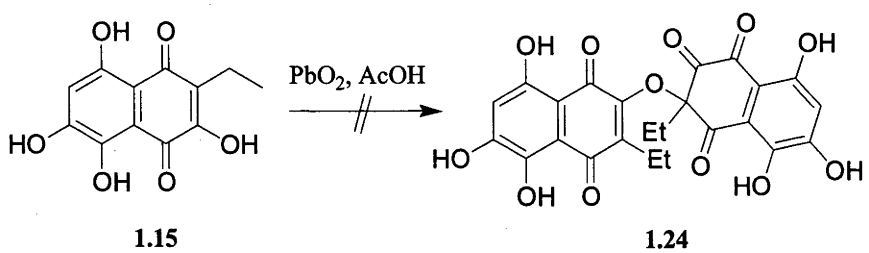
*Scheme 4.12 Lapachol (**1.6**) dimerisation with lead(IV) oxide*

Interestingly, during investigations into the synthesis of islandoquinone (**1.24**), Stepanenko *et al.* treated the naphthazarin **4.15** with lead(IV) oxide in glacial acetic acid at 100°C and the C-O dimer naphthazarin **4.16** was isolated in 62% yield (Scheme 4.13).[1]



Scheme 4.13 Stepanenko et al. *dimerisation of naphthazarin **4.15** with lead(IV) oxide*

In particular, the C-O dimer **4.16** was structurally similar to the naturally occurring *bis*-naphthazarin, islandoquinone (**1.24**). We therefore anticipated that treatment of 3-ethyl-2,7-dihydroxynaphthazarin (**1.15**) with lead(IV) oxide in acetic acid should result in the synthesis of islandoquinone (**1.24**). However, a complex mixture of products was obtained when we attempted to synthesise islandoquinone (**1.24**) in this manner. The reaction was repeated several times but the reaction outcome was consistently complex (Scheme 4.14).



*Scheme 4.14 Attempted synthesis of islandoquinone (**1.24**)*

When phthiocol (**1.7**), however, was treated with lead(IV) oxide in hot glacial acetic acid for ten minutes, HPLC analysis of the reaction mixture indicated the formation of two major products that could be separated chromatographically (Figure 4.4). A white, crystalline solid (**4.17**), with a retention time of 16 minutes on reverse-phase HPLC, was isolated along with a yellow solid (**4.18**) with a retention time of 21 minutes. When the reaction time for treatment with lead(IV) oxide was increased to 18 hours the yellow compound was the only product isolated.

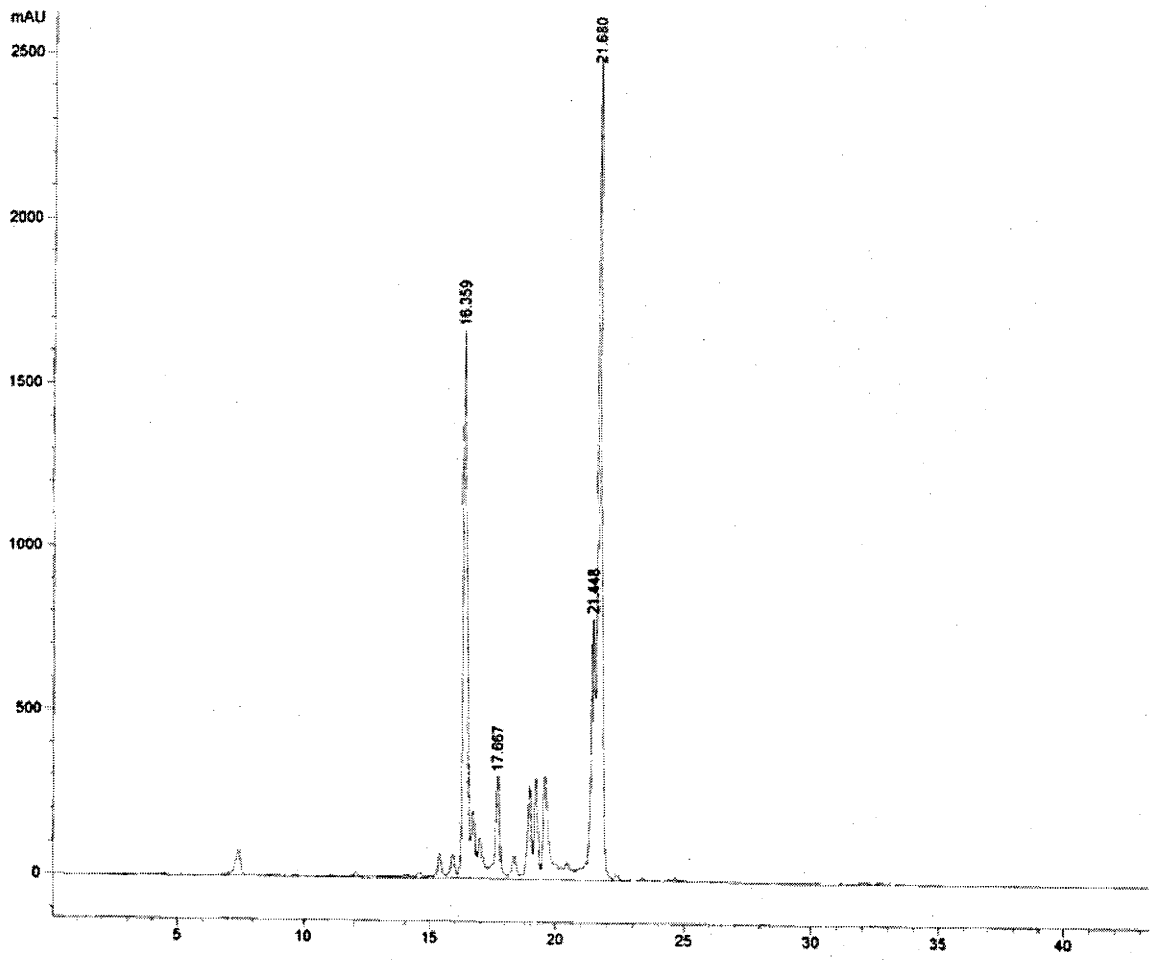


*Figure 4.4 Absorbance (mAU) versus Retention Time (min): treatment of phthiocol (**1.7**) with lead(IV) oxide for 10 minutes*

The proton NMR spectrum of the white crystalline solid (**4.17**) displayed two methyl singlets at 1.41 ppm and 1.78 ppm, indicating the presence of distinct methyl environments within the molecule. Multiplets in the aromatic region from 7.29 ppm to 8.05 ppm suggested that the aromatic moieties remained intact. Two broad singlets were also present at 5.78 ppm and 6.61 ppm. These singlets were exchangeable in D_2O as expected for acidic hydroxy functionalities.

The carbon NMR spectrum of derivative **4.17** contained 22 distinct carbon signals and this supported the idea that the compound was dimeric in nature. Significantly, only two carbonyl carbon signals were observed, at 183.5 ppm and 187.5 ppm. In addition, eight aromatic *C*H signals were evident due to the intact aromatic moieties, along with two methyl resonances at 6.5 ppm and 14.5 ppm.

A molecular ion at *m/z* 380 was observed in the low-resolution electron impact mass spectrum. The fragmentation pattern showed a loss of two hydroxy groups to give rise to an ion at *m/z* 346. As we were unable to unambiguously identify the structure through these spectroscopic techniques, a single crystal of this compound was grown in chloroform and the X-ray crystallographic analysis elucidated the structure as shown in Figure 4.5. A solution of the crystal sample was analysed by HPLC to establish its purity and verify the identity of the crystal as that of the compound with a retention time of 16 minutes on reverse-phase HPLC.

Derivative **4.17** crystallised from chloroform as colourless prisms. The structure comprises one molecule of derivative **4.17** and one molecule of chloroform per asymmetric unit. The bond lengths and angles were in agreement with typical values. A complex hexacyclic structure is evident, with two hemiacetal linkages.

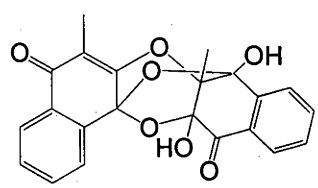


Figure 4.5a Derivative 4.17

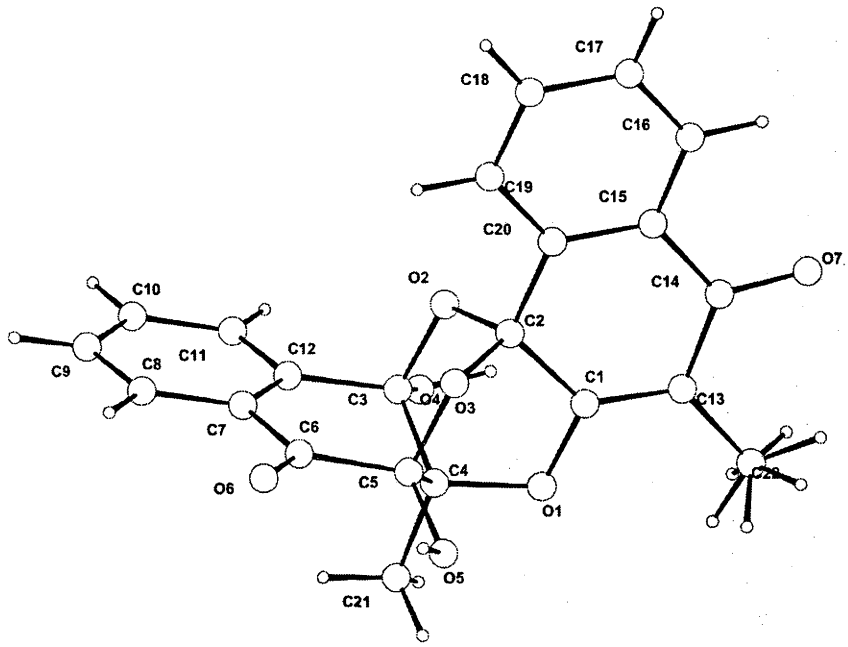
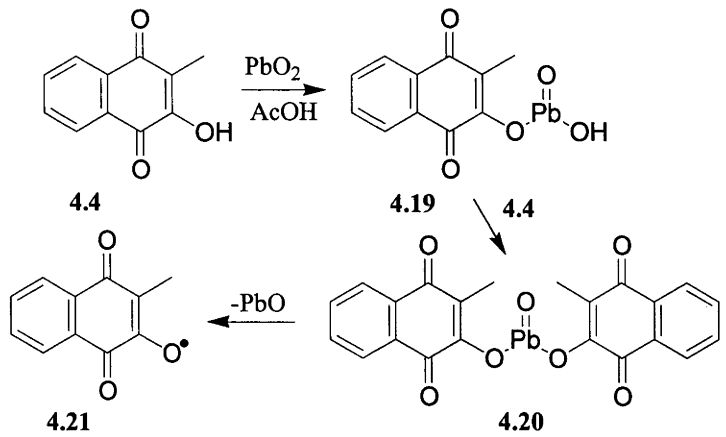


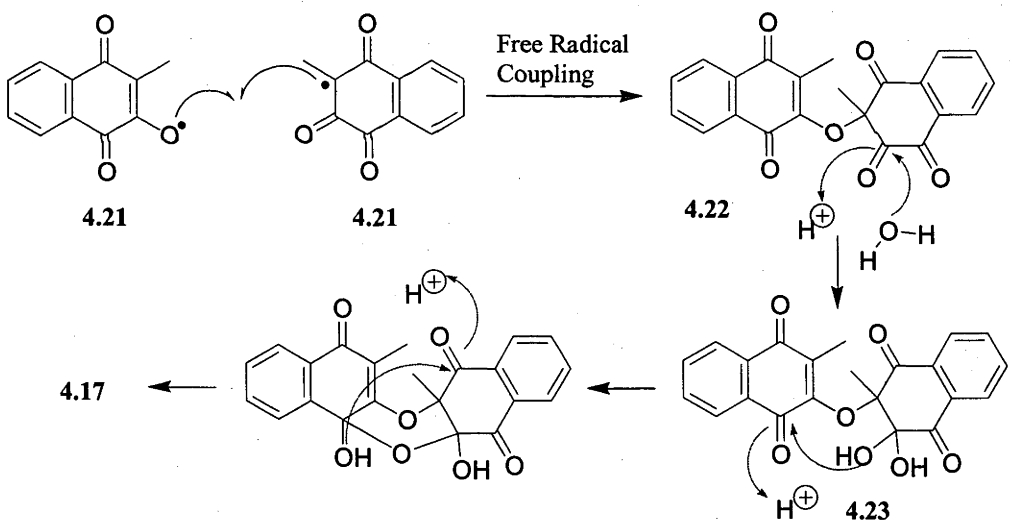
Figure 4.5b Thermal ellipsoid diagram of the bis-*naphthoquinone derivative* ***4.17*** *with selected atom labelling. Ellipsoids show 50% probability levels. Hydrogen atoms are drawn as circles with small radii. (X-ray analysis performed by Dr Alison Edwards)*

The mechanism for the formation of this novel dimer **4.17** presumably involves the generation of intermediate alkoxy radical species, in an analogous fashion to those formed during phenolic coupling reactions under these conditions.[36] Thus, the lead(IV) complex **4.19** formed initially would complex with another molecule of phthiocol (**1.7**) to give the lead(IV) dimer **4.20**. Decomposition of **4.20** then gives rise to the radical species **4.21** with concomitant loss of lead(II) monoxide (Scheme 4.15).



Scheme 4.15 Formation of radical ***4.21*** *using lead(IV) oxide*

Radical coupling then leads to the formation of the C-O dimer **4.22**, which can undergo nucleophilic attack by water to give the *gem*-diol **4.23**. Two sequential intramolecular nucleophilic attacks to the appropriate carbonyl carbon atoms would then lead to the formation of the observed product **4.17** (Scheme 4.16).



Scheme 4.16 Proposed mechanism for the formation of derivative ***4.17***

It then remained to determine the structure of the yellow derivative **4.18**. The proton NMR spectrum of this compound contained two distinct methyl signals at 1.72 ppm and 2.24 ppm and aromatic protons were evident as multiplets in the region 7.45 ppm to 8.02 ppm. The appearance of two methyl signals again suggested that the compound was dimeric in nature. Analysis of the low-resolution electrospray mass spectrum supported this hypothesis, as a

molecular ion was observed at *m/z* 347, the high-resolution analysis of which gave an elemental composition of $C_{21}H_{14}O_5$.

The carbon NMR of this compound, however, indicated the presence of just 16 carbon environments, suggesting that the structure contained some degree of symmetry. Three carbonyl carbon signals were observed at 181.1 ppm, 185.0 ppm and 195.7 ppm and two methyl resonances were observed at 9.8 ppm and 23.5 ppm. The methyl signal at 23.5 ppm is significantly downfield, suggesting that this carbon is in a distinctly different environment to the methyl groups of both phthiocol (**1.7**) and derivative **4.17**. The two upfield carbonyl signals are consistent with naphthoquinone carbonyl carbon resonances. The presence of a quaternary carbon signal at 84.1 ppm also suggested that a new structural feature was present in the derivative **4.18**, in comparison with phthiocol (**1.7**) and derivative **4.17**.

The infrared spectrum of derivative **4.18** indicated the presence of three carbonyl groups, with absorptions at 1759 cm^{-1} and two characteristic naphthoquinone type carbonyl groups at 1724 and 1658 cm^{-1}. The yellow colour of derivative **4.18** was indicative of conjugation within the molecule. This evidence tentatively suggested to us that an intact naphthoquinone moiety was present in the structure of **4.18**.

An attempt was then made to elucidate the structure of derivative **4.18** via a two-dimensional gHMBC NMR experiment and this further supported the presence of a naphthoquinone moiety (Figure 4.6). A $^1J_{CH}$ correlation was observed between the methyl protons at 2.24 ppm and the methyl carbon at 9.8 ppm, a $^3J_{CH}$ correlation was observed between these protons and the carbon at 152.8 ppm, $^3J_{CH}$ coupling was evident with the carbonyl carbon at 185.0 ppm and, finally, a $^4J_{CH}$ correlation was observed between these protons and the other remaining carbonyl carbon at 181.1 ppm.

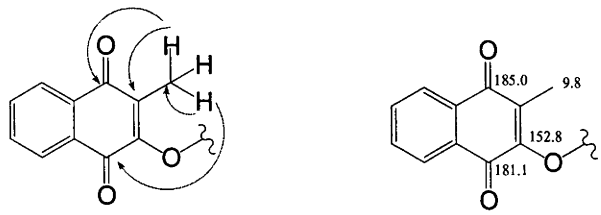


Figure 4.6 The gHMBC correlations and assignments for the naphthoquinone fragment

Eleven of the carbons present were therefore accounted for with the interpretation of the naphthoquinonoid fragment through analysis of the gHMBC spectrum and comparison with the carbon NMR spectrum of phthiocol (**1.7**). In addition, a $^{1}J_{CH}$ correlation was observed between the methyl protons at 1.72 ppm and the methyl carbon at 23.4 ppm, a $^{2}J_{CH}$ correlation was observed between these protons and the quaternary carbon at 84.1 ppm and a $^{3}J_{CH}$ correlation was observed between these protons and the carbonyl group at 195.7 ppm.

A single crystal of this compound was grown in chloroform and the structure was confirmed to be 2-methyl-3-(2-methyl-1,3-dioxo-indan-2-yloxy)-1,4-naphthoquinone (**4.18**) (Figure 4.7). Structures of this type have been synthesised previously through the reaction of indane-1,3-diones with naphthoquinones,[37-39] however, to the best of our knowledge, this is the first example of an indane-1,3-dione synthesis through the oxidative dimerisation of a naphthoquinone.

Derivative **4.18** crystallised from ethyl acetate as yellow prisms and the structure contains a large solvent void occupied by highly disordered ethyl acetate molecules. Each asymmetric unit contains two molecules of derivative **4.18** along with one molecule of ethyl acetate. The bond lengths and angles observed were within the normal range. The identity of the crystals was ascertained via HPLC analysis to be that of the compound with the retention time of 21 minutes by reverse-phase HPLC.

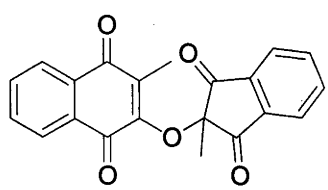


*Figure 4.8a 2-Methyl-3-(2-methyl-1,3-dioxo-indan-2-yloxy)-1,4-naphthoquinone (**4.18**)*

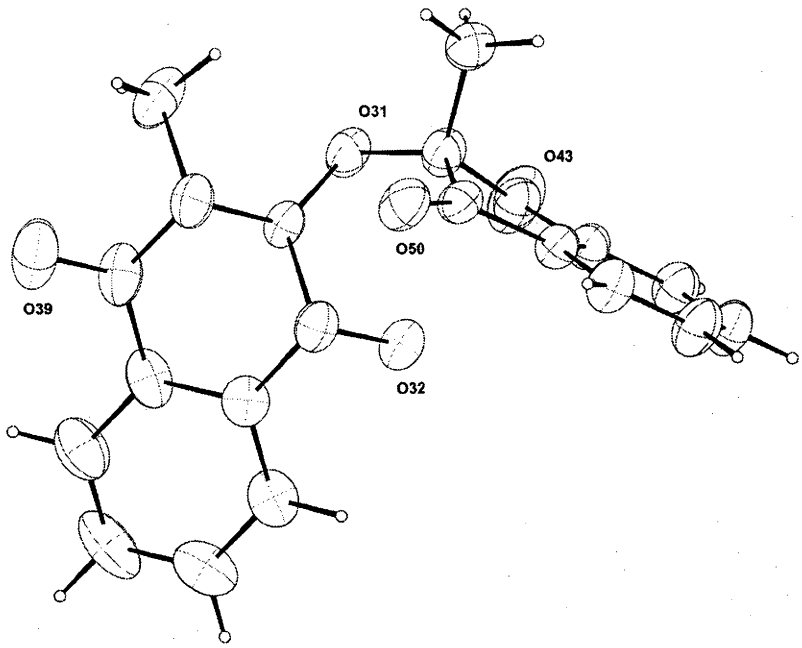
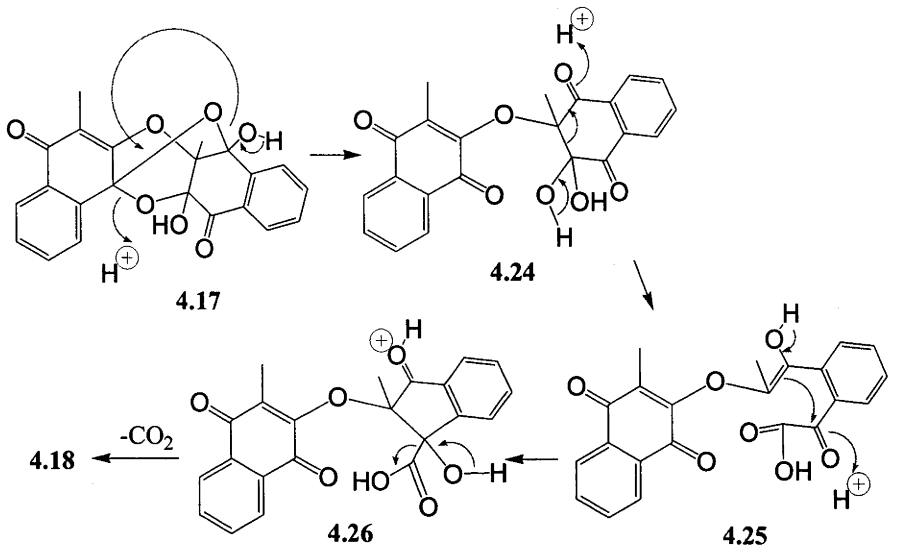


Figure 4.8b Thermal ellipsoid diagram of the indane-1,3-dione derivative ***4.18*** *with selected atom labelling. Ellipsoids show 50% probability levels. Hydrogen atoms are drawn as circles with small radii. (X-ray analysis performed by Dr Alison Edwards)*

During the course of this investigation, it was observed that the dimer **4.17** decomposes to give the yellow derivative **4.18** in the absence of lead(IV) oxide and glacial acetic acid. This suggested that the indane-1,3-dione derivative **4.18** arises from the hexacyclic derivative **4.17**. The proposed mechanism for this process is outlined in Scheme 4.17. Derivative **4.17** may undergo sequential hemiacetal-acetal ring cleavage to give rise to the derivative **4.24**, which can further fragment to give the carboxylic acid **4.25**. The formation of the five-membered ring then gives rise to derivative **4.26**, the decarboxylation of which would afford derivative **4.18**.



Scheme 4.17 Proposed mechanism for the formation of indane-1,3-dione derivative ***4.18***

While these unusual phthiocol dimers are inherently interesting from a structural and mechanistic viewpoint, they did not provide us with further insight into the dimerisation of naphthazarins, as analogous reactions were not observed when 3-ethyl-2,7-dihydroxynaphthazarin (**1.15**) was treated with lead(IV) oxide in glacial acetic acid. We then continued to investigate the use of alternative oxidants for the dimerisation of naphthazarins.

4.2.6 Attempted Synthesis of Bis-*Naphthazarins using Alternative Oxidants*

Ceric ammonium nitrate (CAN) has been utilised successfully for both the deprotection of methyl ethers and the dimerisation of quinones.[40,41] When lawsone (**1.3**) was treated with CAN, under the conditions employed by Jacob *et al.* for benzoquinone dimerisation, only 10% conversion to bilawsone (**4.5**) was observed together with the formation of undesired by-products. The treatment of diethylmompain **2.53** under identical conditions only resulted in the recovery of unreacted starting material. Interestingly, when phthiocol (**1.7**) was subjected to these reaction conditions the naphthoquinone derivatives **4.17** and **4.18** were evident in the HPLC trace of the reaction mixture (Entry 1, Table 4.1). These conditions were not optimized for the synthesis of derivatives **4.17** and **4.18**.

Potassium ferricyanide has often been used for the oxidative dimerisation of phenolic substrates[42], but when we treated lawsone (**1.3**), phthiocol (**1.7**) or diethylmompain (**2.53**) with potassium ferricyanide no reaction was observed (Entry 2, Table 4.1). Similarly, manganese(IV) oxide is known to affect the dimerisation of phenolic substrates through a one-electron oxidation.[43] Somewhat disappointingly, a complex mixture of products was obtained for both lawsone (**1.3**) and diethylmompain (**2.53**) when manganese(IV) oxide was employed (Entry 3, Table 4.1).

Table 4.1 Dimerisation Studies Under Oxidative Conditions

Entry	Reagent(s)	Starting Material	Observations*
1	CAN	Lawsone (**1.3**) Diethylmompain (**2.53**) Phthiocol (**1.7**)	10% conversion to **4.5** Starting material only **4.17** and **4.18** evident
2	Potassium Ferricyanide	Lawsone (**1.3**) Phthiocol (**1.7**) Diethylmompain (**2.53**)	Starting material only Starting material only Starting material only
3	Manganese(IV) Oxide	Lawsone (**1.3**) Diethylmompain (**2.53**)	Complex Reaction Mixture Complex Reaction Mixture
4	Silver(I) Oxide/ Nitric Acid	Lawsone (**1.3**)	Traces of **4.5** evident
5	Ferric Chloride	Lawsone (**1.3**) Phthiocol (**1.7**)	Starting material only Starting material only
6	Horseradish Peroxidase	Lawsone (**1.3**) Diethylmompain (**2.53**) Boryquinone (**1.16**)	24% conversion to **4.5** Starting material only Starting material only

* HPLC analysis was used to monitor these studies.

The use of silver(II) oxide with nitric acid has recently been reported by Tanoue *et al.* for the dimerisation of naphthols.[44] The dimerisation of lawsone (**1.3**) to bilawsone (**4.5**) under these conditions proceeded in very low yield and so the use of these reagents was not explored further (Entry 4, Table 4.1). Finally, the use of ferric chloride was examined

following the procedure developed by Hooker.[45] When both lawsone (**1.3**) and phthiocol (**1.7**) were treated with aqueous ferric chloride, however, only starting material was recovered (Entry 5, Table 4.1).

As the biosynthesis of dimeric naphthoquinones is presumably under enzymatic control, we also examined the use of commercially available horseradish peroxidase.[46,47] When lawsone (**1.3**) was treated with the enzyme in a buffered solution at 37°C, formation of bilawsone (**4.5**) proceeded with 24% conversion after one week. However, both diethylmompain (**2.53**) and boryquinone (**1.16**) failed to react under these conditions (Entry 6, Table 4.1). As the formation of bilawsone (**4.5**) may be due to a photolytic reaction in the laboratory, when lawsone (**1.3**) was allowed to react with horseradish peroxidase under analogous conditions with the exclusion of light, only 8% conversion to bilawsone (**4.5**) was observed. This suggests that the photochemical behaviour of lawsone (**1.3**) may account for the formation of some of the bilawsone (**4.5**) and that the enzyme is not efficient in converting lawsone (**1.3**) into bilawsone (**4.5**) under these conditions.

It was therefore evident that while the C-C dimerisation of 2-hydroxy-1,4-naphthoquinone (lawsone, **1.3**) was readily achieved under a range of conditions, the analogous reactions of 3-alkyl-2-hydroxy-1,4-naphthoquinones remains poorly understood. This observation also applied to the naphthazarins under investigation, as either starting material was recovered or a complex mixture of products was obtained. Further investigations are therefore required to elucidate the behaviour of naphthazarins under oxidative conditions, with a view to the synthesis of *bis*-naphthazarin derivatives.

4.3 Conclusions

The above results indicate that there are many factors that influence the reactions of naphthoquinones with oxidants. The obvious differences between the reactivity of lawsone (**1.3**) and phthiocol (**1.7**) must be attributed to the presence of the *C*-methyl substituent in the latter. The conditions required for the dimerisation of highly functionalised naphthazarins, such as boryquinone (**1.16**), differ significantly from those suitable for the dimerisation of less complex analogues. Whilst we were unable to successfully synthesise the desired *bis*-naphthazarin derivatives, the dimerisation of lawsone (**1.3**) was investigated

under a variety of conditions and reagents and two novel dimers were accessed synthetically from phthiocol (**1.7**).

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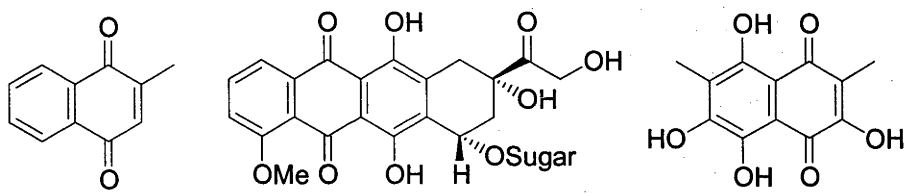
Chapter Five:

Evaluation of Biological Activity

5.1 Introduction

The National Cancer Institute of the United States has identified the quinone structural motif as a recurrent pharmacophore in cytotoxic compounds.[1] Quinones have also been widely recognised for their antibacterial and anti-malarial activity and are therefore of considerable interest to medicinal chemists. An important subclass of quinones consists of compounds containing the naphthoquinone moiety, including several pharmaceutical agents in clinical use today. For example, 2-methyl-1,4-naphthoquinone (menadione, **1.2**) has been shown to induce single and double strand DNA breaks in human MCF-7 cells due to the production of reactive oxygen species. Menadione (**1.2**) may be also valuable when used in chemotherapy in conjunction with radiotherapy for the treatment of human malignancies.[2,3]



1.2 **5.1** **1.14**

Where Sugar = daunosamine

Figure 5.1 Menadione (1.2), adriamycin (5.1) and aureoquinone (1.14)

More structurally complex naphthoquinones have also been shown to display potent cytotoxicity. Adriamycin (**5.1**) has been isolated from *Streptomyces peucetius* and is currently used to treat breast and esophagel carcinomas, osteosarcoma, Kaposi's sarcoma, soft-tissue sarcoma and Hodgkin's and non-Hodgkin's lymphomas. Adriamycin (**5.1**) is also effective against gastric, liver, bile duct, pancreatic and endometrial carcinomas and displays antibiotic activity.[4]

Given the established medicinal value of naturally occurring naphthoquinones, the isolation of novel naturally occurring naphthoquinones is of interest. Berg *et al.* have recently isolated aureoquinone (**1.14**) from surface cultures of *Aureobasidium* sp. grown on a synthetic medium (Figure 5.1). Aureoquinone (**1.14**) was shown to display moderate anti-microbial activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC: 120 μgmL^{-1}). The inhibition of a number of protease enzymes was also shown via dye labelled casein substrate assays (Table 5.1).[5]

*Table 5.1 Aureoquinone (**1.14**) induced protease inhibition*

Protein utilised in Assay	IC_{50} (μgmL^{-1})[#]
Trypsin	11.4
Papain	14.5
Thermolysin	17.8
Collagenase	7.1
Zinc-protease	8.7

The isolation of hybocarpone (**1.25**), a naturally occurring *bis*-naphthazarin derivative, by Elix *et al.* therefore instigated the preliminary examination of its biological behaviour (Figure 5.2). The antiproliferative activity of hybocarpone (**1.25**) was measured against the murine P815 mastocytoma cell line via a radiolabelled thymidine incorporation assay. Hybocarpone (**1.25**) displayed potent cytotoxicity, with an IC_{50} value of 0.27 μM.[6]

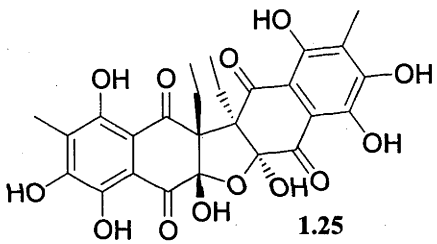


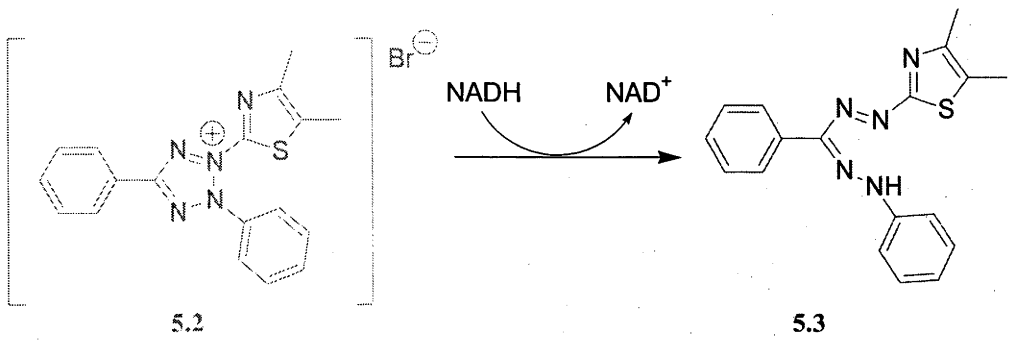
*Figure 5.2 Hybocarpone (**1.25**) and boryquinone (**1.16**)*

As hybocarpone (**1.25**) comprises a novel dimeric naphthazarin structure, the pharmacophore that gives rise to its cytotoxicity remains to be identified. Boryquinone (**1.16**) is the putative biosynthetic precursor to hybocarpone (**1.25**) and the synthesis of this naphthazarin is described in Chapter Two. Our initial hypothesis was that the bioactivity of hybocarpone (**1.25**) could be due to the *in vitro* formation of boryquinone (**1.16**). As boryquinone (**1.16**) contains a quinonoid structural core, cell death could conceivably occur via redox cycling and/or be due to the ability of quinones to react with cellular constituents. We therefore embarked on biological studies to compare the bioactivity of hybocarpone (**1.25**) with that of boryquinone (**1.16**). In addition, the biological behaviour of structurally related synthetic aureoquinone (**1.14**) was also examined.

[#] The IC_{50} is defined as the concentration of compound required to inhibit cell proliferation by 50%.

5.2 Evaluation of Antiproliferative Activity

The colourimetric MTT assay is one of the safest and simplest methods for measuring cell viability. The MTT assay involves the reduction of the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, **5.2**) to the dark purple formazan derivative **5.3** in metabolically active cells by biological reducing equivalents such as nicotinamide adenine dinucleotide (NADH). Cells that are not metabolically active cannot reduce MTT (**5.2**) to the formazan derivative **5.3** and so the yellow colour persists after incubation (Scheme 5.1). The number of metabolically active cells can then be quantified via ultraviolet/visible analysis.[7,8]



5.2 5.3

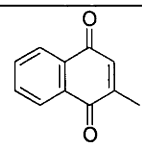
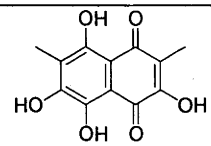
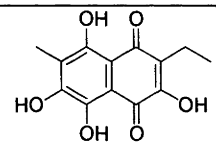
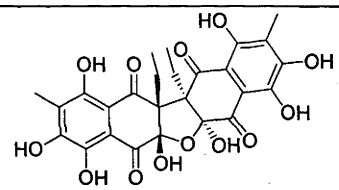
*Scheme 5.1 Reduction of MTT (**5.2**) to formazan **5.3** by NADH*

The antiproliferative activity of the compounds tested was measured against HeLa cells over a range of incubation times, cell densities and toxin concentrations and a suitable protocol was thereby developed. This method was then applied to each of the compounds and an IC_{50} value was determined from a semi-logarithmic plot of concentration against absorbance at 590nm (A_{590}), in which non-linear regression analysis was used to fit a sigmoidal concentration-response curve through the data points. The GraphPad Prism 2.0 software package was used to fit the curves from quadruplicate sets of data and to calculate the corresponding IC_{50} values.

The results for the quinones tested are presented in Table 5.2. Menadione (**1.2**) was also employed in this investigation due to its well-established bioactivity. The cells treated with the highest concentration of menadione (**1.2**) [100 μM] were as metabolically active as untreated control cells after an overnight incubation period but weak activity was observed when the incubation period was extended to three days (IC_{50} 84.8 μM). The cytotoxicity of menadione (**1.2**) against a range of human cancer cell lines has recently been reported by Wu *et al.* and the average IC_{50} values obtained ranges from

15-42 μM.[9] It is not clear why the value obtained in this assay differs from reported values but a number of factors, such as the incubation period or cell density, conceivably contribute to this result.

Table 5.2 IC_{50} values determined for activity against HeLa Cells

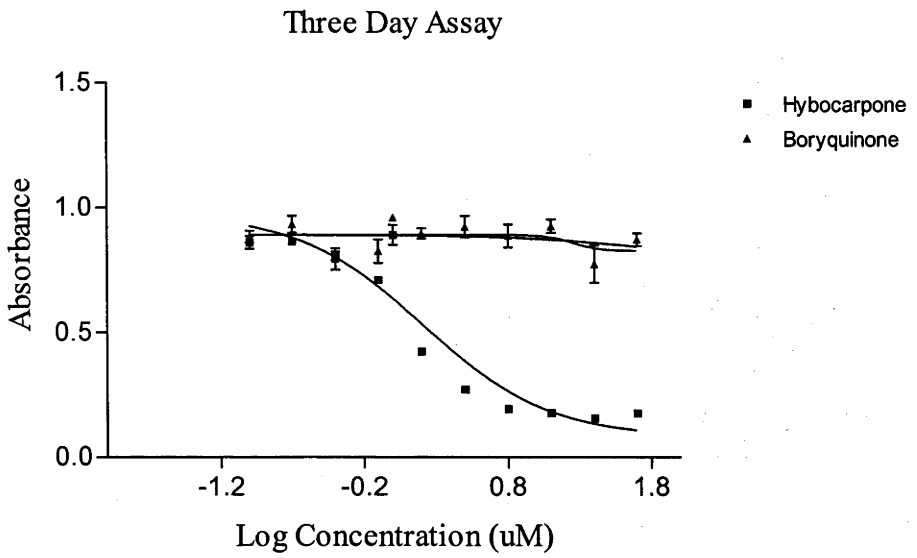
Compound	Structure	Incubation period (days)	IC_{50} (μM)
Menadione **(1.2)**		1 3	>100 84.8 ± 0.40
Aureoquinone **(1.14)**		3 7	>100 >100
Boryquinone **(1.16)**		3 7	>100 48.3 ± 0.10
Hybocarpone **(1.25)**		1 3 7	3.7 ± 0.03* 1.1 ± 0.10 3.6 ± 0.02*

* These values were obtained using an aged solution of hybocarpone **(1.25)**

Synthetically derived aureoquinone **(1.14)** was not found to inhibit the proliferation of HeLa cells to a measurable extent within the parameters of the MTT assay. Despite the structural similarity between aureoquinone **(1.14)** and boryquinone **(1.16)**, moderate bioactivity was observed for boryquinone **(1.16)** after a seven day incubation period (IC_{50} value 48.3 μM).

On the other hand, it was evident that hybocarpone **(1.25)** elicits a typical toxin dose-response effect on the HeLa cells. A typical dose-response curve is shown in Graph 5.1. Hybocarpone **(1.25)** displayed potent cytotoxicity against HeLa cells after an incubation period of three days (IC_{50} value 1.1 μM) and this is comparable to the activity observed previously against the P388 cell line measured using a thymidine incorporation assay (IC_{50} value 0.27 μM). As boryquinone **(1.16)**, however, was found to be significantly

less active than hybocarpone (**1.25**), the biological activity of the latter cannot be simply attributed to the formation of the monomeric naphthazarin, boryquinone (**1.16**).



Graph 5.1 Dose-response curves for hybocarpone (1.25) and boryquinone (1.16)

Although the initial three day assay indicated that hybocarpone (**1.25**) has an IC_{50} value of 1.1 μM. We then used the stock solution of hybocarpone (**1.25**), which had been stored at -85°C for four days, to perform two more assays, the first involved an incubation period of eighteen hours and the second involved an incubation period of one week. The IC_{50} values obtained for these assays were 3.7 μM and 3.6 μM respectively. The similarity in these IC_{50} values suggests that the observed bioactivity may be due to biological events that occur within the first eighteen hours of exposure to the hybocarpone (**1.25**) solution.

The decrease in the IC_{50} values obtained warrants further investigation. In view of the hemiacetal nature of hybocarpone (**1.25**), it is possible that the biological activity may relate to the stability of the dimeric naphthazarin. Our preliminary observation concurred with the proposal that hybocarpone (**1.25**) is not stable under the conditions employed in the MTT assay. In particular, a reverse phase HPLC analysis of the stock solution of hybocarpone (**1.25**) used for the biological tests indicated that significant amounts of boryquinone (**1.16**) were present (Figure 5.3). The ultraviolet spectrum of each component from the HPLC trace was analysed using a photodiode array detector and the identity of boryquinone (**1.16**) and hybocarpone (**1.25**) was determined from a database of compounds.[10] The presence of boryquinone (**1.16**) in the aged stock

solutions of hybocarpone (**1.25**) prompted a more detailed investigation into the decomposition of hybocarpone (**1.25**).

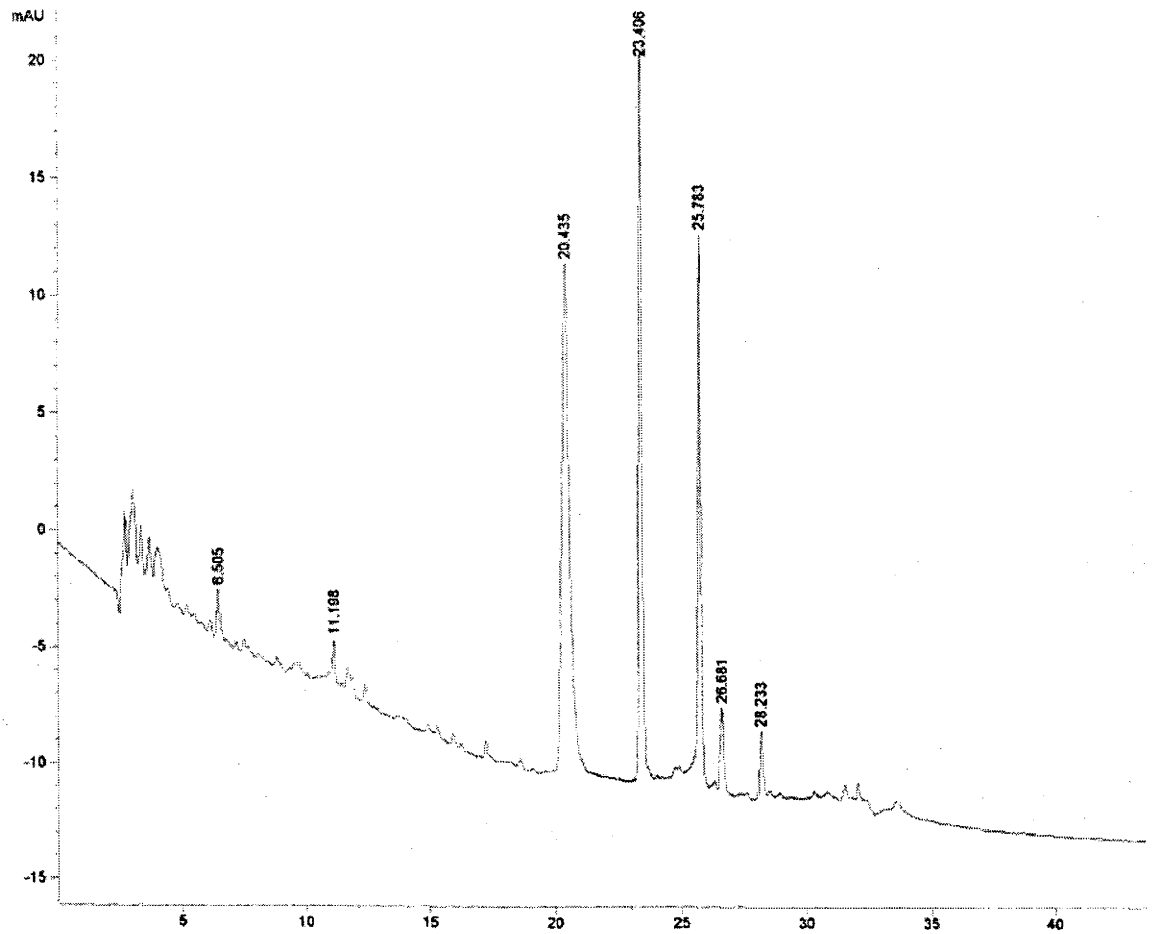


*Figure 5.3 HPLC trace of stock solution of hybocarpone (**1.25**) used for overnight incubation assay (IC_{50} value 3.7 μM, pH = 7.6). Hybocarpone (**1.25**) is evident at 25.8 mins, boryquinone (**1.16**) is evident at 23.4 mins and the remaining peaks are unidentified.*

Given that the purity of hybocarpone (**1.25**) used in these assays had been previously established spectroscopically and through HPLC analysis (Figure 5.4), the appearance of boryquinone (**1.16**) in the stock solution is presumably due to the degradation of hybocarpone (**1.25**).

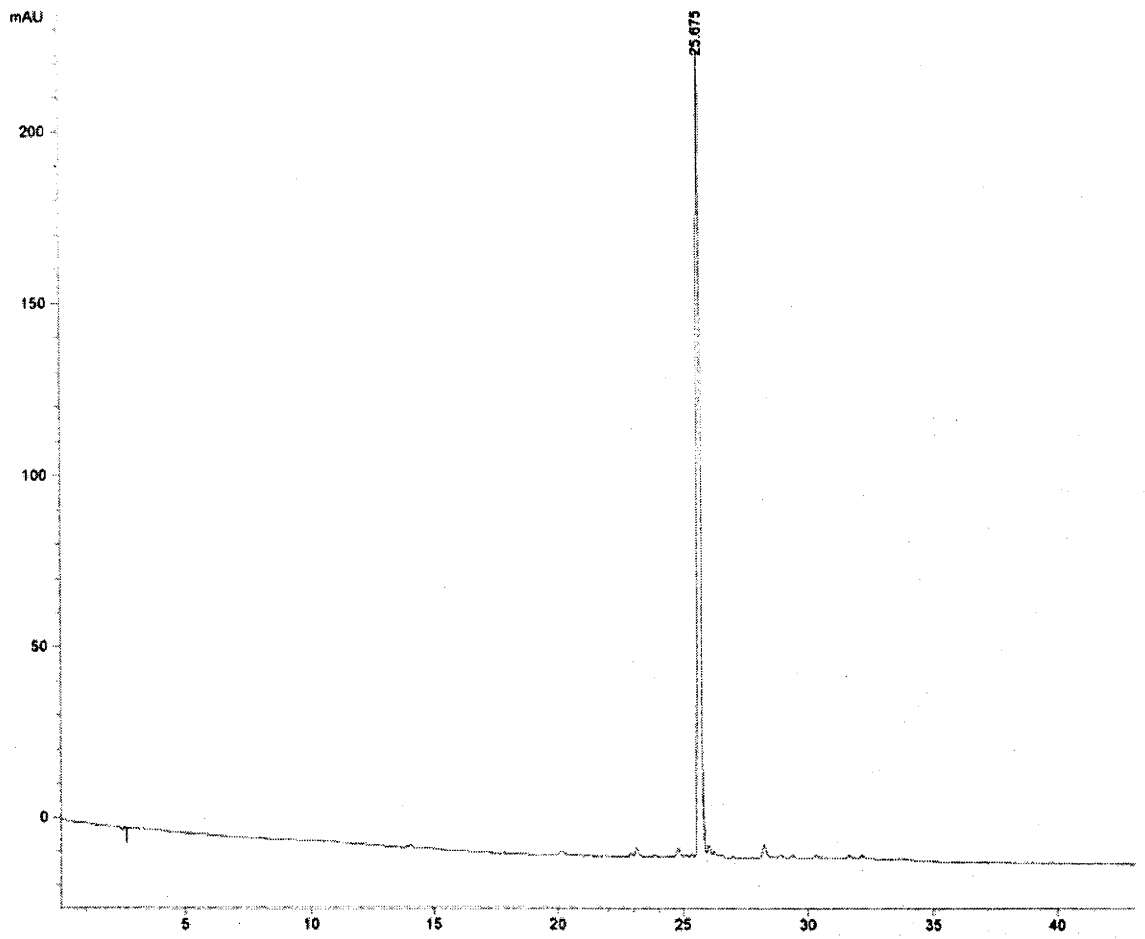


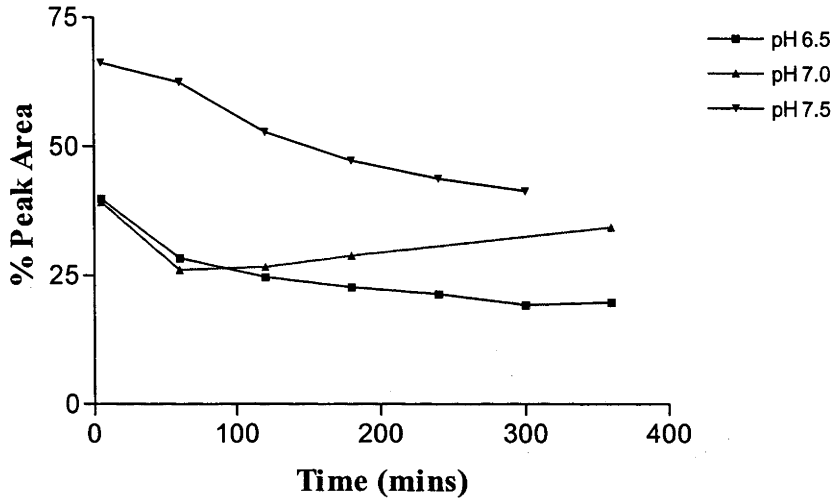
*Figure 5.4 The HPLC trace of hybocarpone (**1.25**) utilised to prepare stock 500 μM solution and in stability studies*

*5.3 Stability of Hybocarpone (**1.25**)*

A known quantity of hybocarpone (**1.25**) was dissolved in 100mM 2-amino-2-(hydroxymethyl)-1,3-propandiol (TRIS) buffer at pH 6.5, 7.0 and 7.5. The solutions were monitored at 290nm for hybocarpone (**1.25**) and boryquinone (**1.16**) by reverse phase HPLC at various time intervals. The solutions were incubated throughout the experiment at 37.5°C in a carbon dioxide atmosphere so as to mimic the conditions employed during the biological assay. The initial pH of the media solution of hybocarpone (**1.25**) used for the biological tests was measured to be 7.6.

The results obtained for the first six hours are represented in Graph 5.2. The % peak area refers to the percent of the total peak area attributable to hybocarpone (**1.25**) and a reduction in the % peak area is therefore indicative of a decrease in the concentration of hybocarpone (**1.25**). The first time point was measured five minutes after the solutions were made up. Given the notable reduction in % peak area after five minutes, it is

evident that hybocarpone (**1.25**) was unstable in all three aqueous TRIS buffer solutions, as identical starting concentrations were used. This effect is less pronounced for the pH 7.5 hybocarpone (**1.25**) solution, however, significant instability was also observed at this pH value.



Graph 5.2 The Degradation of hybocarpone (1.25) at various pH values

These stability studies also indicated that the hybocarpone (**1.25**) degradation process is complex, as a number of unidentified compounds were evident via HPLC analysis. Although an increase in the concentration of boryquinone (**1.16**) was observed within the first six hours, a complex mixture of products was observed at all three pH values after an overnight incubation. When boryquinone (**1.16**) was subjected to an identical analysis, it was stable within the timeframe of the experiment.

The stability of hybocarpone (**1.25**) in other solvents at room temperature was also qualitatively examined (Table 5.3). Hybocarpone (**1.25**) was dissolved in the appropriate solvent and the solution was monitored over time by HPLC analysis. This investigation indicated that hybocarpone (**1.25**) is stable in methanol, acetonitrile and dimethyl sulfoxide (Entries 1, 2, 3 and 4, Table 5.3). As the instability of hybocarpone (**1.25**) may be pH-dependent, anhydrous acids were added to methanolic solutions of the natural product (Entries 5 and 6, Table 5.3). Interestingly, despite the protic and potentially nucleophilic nature of methanol, hybocarpone (**1.25**) was found to be stable under these conditions. The stability of hybocarpone (**1.25**) under anhydrous basic conditions was not recorded. Conversely, hybocarpone (**1.25**) was found to be unstable in any aqueous solution and the presence of boryquinone (**1.16**) was detected within one hour (Entries 7, 8 and 9, Table 5.3).

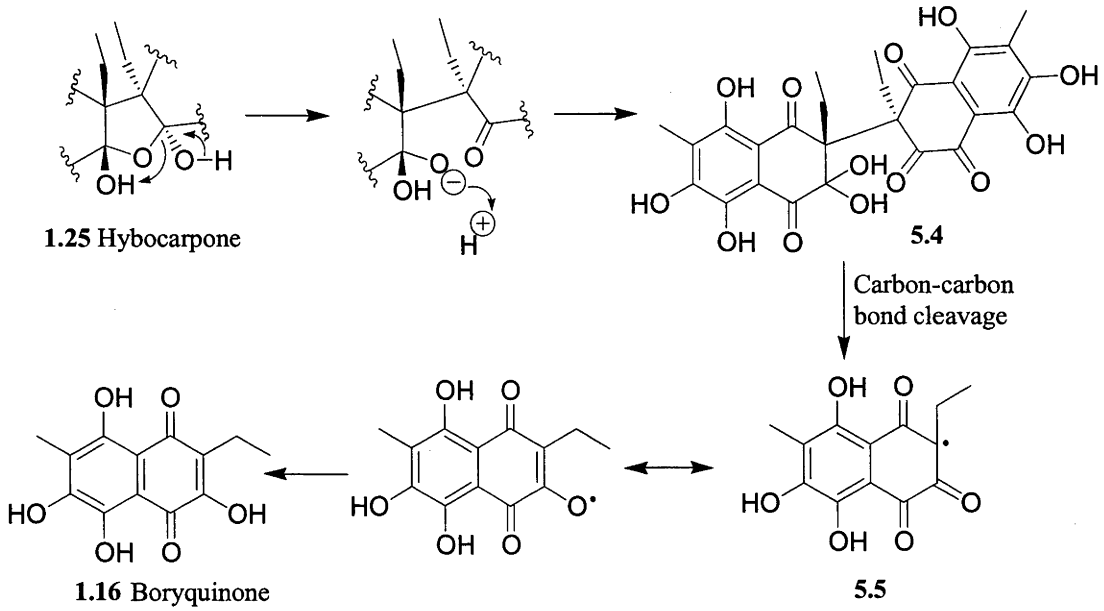
*Table 5.3 Stability of hybocarpone (**1.25**) in solution*

Entry	Solvent(s)	Stability
1	Acetonitrile	+
2	acetonitrile + methanol	+
3	Methanol	+
4	Dimethyl sulfoxide	+
5	methanol + acetic acid (pH 5.05)	+
6	methanol + sulfuric acid	+
7	acetonitrile + water	–
8	media solution (pH 7.6)	–
9	distilled water	–

Where + = stable and - = not stable

Collectively, these studies indicated that hybocarpone (**1.25**) is not stable in aqueous solutions and that boryquinone (**1.16**) and other compounds are formed within minutes. The observed bioactivity of hybocarpone (**1.25**) against HeLa cells decreased over time, when an aged solution of hybocarpone (**1.25**) was employed, and this may have been due to a decrease in the concentration of hybocarpone (**1.25**).

A proposed mechanism for this process is detailed in Scheme 5.2. Hybocarpone (**1.25**) presumably undergoes water-mediated hemiacetal ring-opening to give rise to the gem-diol **5.4**. In aqueous solutions, hybocarpone (**1.25**) may exist in equilibrium with the tetracyclic dimer **5.4**. Homolytic carbon-carbon bond cleavage may then lead to the formation of a monomeric radical species such as **5.5**, which can abstract hydrogen to form boryquinone (**1.16**).



*Scheme 5.2 Proposed mechanism for the formation of boryquinone (**1.16**) under aqueous conditions*

A complete understanding of the factors responsible for the instability of hybocarpone (**1.25**) may lead to an understanding of the reverse process required for the formation of hybocarpone (**1.25**). The number of compounds present in the HPLC traces of aqueous solutions of hybocarpone (**1.25**), however, suggests that this process is complex and may involve the formation of a number of intermediate species. We were not able to convert boryquinone (**1.16**) into hybocarpone (**1.25**) by adjusting the pH of aqueous solutions of boryquinone (**1.16**). This observation is not surprising, as the dimerisation of boryquinone (**1.16**) to hybocarpone (**1.25**) would involve an intermolecular reaction as opposed to the intramolecular process for the degradation of hybocarpone (**1.25**) (Scheme 5.2).

5.4 Conclusions

The antiproliferative activity of hybocarpone (**1.25**), boryquinone (**1.16**), aureoquinone (**1.14**) and menadione (**1.2**) against the HeLa cell line was measured via an MTT assay and the cytotoxicity of hybocarpone (**1.25**) was confirmed through this study. Hybocarpone (**1.25**) was found to be unstable in aqueous solutions and the formation of boryquinone (**1.16**), amongst other unidentified compounds, was confirmed by HPLC analysis.

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Chapter Six: Experimental

6.1 General Procedures for Chapters Two, Three and Four

- Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on a Varian Gemini II NMR spectrometer, operating at 300 MHz for proton and 75.4 MHz for carbon spectroscopy. Deuterochloroform ($CDCl_3$) was used as the solvent unless otherwise indicated. The chemical shifts (δ) are reported as the shift in parts per million (ppm) from tetramethylsilane (TMS, 0.00 ppm). Proton spectra recorded in $CDCl_3$ were referenced to either the residual chloroform singlet (7.24 ppm) or to TMS. Carbon spectra recorded in $CDCl_3$ were referenced to the central peak of the $CDCl_3$ triplet at 77.0 ppm. Proton spectral data are reported as follows: chemical shift (δ), multiplicity (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, dd: doublet of doublets etc., b: broad), relative integral (number of protons), coupling constant (^nJ_x Hz, where n = number of intervening bonds between protons, x = spin system) and assignment where possible. Carbon spectral data are reported as follows: chemical shift (δ) and assignment where possible. Where necessary, the attached proton test (APT) was used to aid in the assignment of carbon NMR data.

- Infrared spectra were recorded on a Perkin Elmer 1800 or a Shimadzu FTIR-8400 Fourier Transform Infrared Spectrometer. Samples were analysed as KBr discs (for solids) or as thin liquid films (for oils) on NaCl plates. Infrared spectral data are reported as follows: frequency (ν_{max} cm^{-1}), strength (vs: very strong, s: strong, m: medium, w: weak).

- Low electron impact mass spectra were recorded on a VG Micromas 7070F double focusing mass spectrometer at 70 eV using positive ion electron impact techniques. Mass spectral data are listed as mass-to-charge ratios (*m/z*) and relative intensity (% of base peak). High-resolution electron-impact mass spectra (HREIMS) were determined on the same instrument by peak matching.

- Melting points are uncorrected and were recorded on a Leica Galen III microscope.

- Microanalyses were performed by the Australian National University Microanalytical Service on a Carlo Erba 1106 CHN-O analyser.

- Analytical thin layer chromatography (TLC) was conducted on aluminium sheets or glass plates coated with silica gel 60 GF_{254} (Merck). The chromatograms were analysed under a 254 nm UV lamp and developed using a reagent 'dipping' solution [ammonium molybdate/ ceric ammonium sulfate/ sulfuric acid/ water (10g: 0.4g: 5.6 mL: 200 mL)] followed by heating.

- Flash chromatography was conducted according to the method of Still and co-workers[1] using Merck silica gel 60 (200-400 mesh ASTM) and analytical reagent (AR) grade solvents indicated.

- Unless otherwise stated, reagents were purchased from Aldrich, Merck or Fluka and used without further purification. Drying agents and other inorganic salts were purchased from AJAX or BDH Chemicals. All solvents were of AR grade, purified by literature procedures and where appropriate, stored over freshly activated molecular sieves.[2]

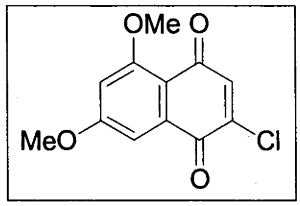
- All reactions were carried out under an atmosphere of dry, oxygen-free nitrogen unless otherwise specified. Reactions that involved moisture sensitive compounds were carried out using oven-dried or flame-dried apparatus and with dry solvents.

- High performance liquid chromatography (HPLC) was performed using a Spectra System, a Phenomenex Hypersil 5C18 column (250 by 4.6 mm) and a spectrometric detector operating at 254 nm with a flow rate of 1 mL/min. Two solvent systems were used: 1% aqueous orthophosphoric acid and spectroscopic grade methanol (7:3) (A) and spectroscopic grade methanol (B). The run started with 100% A and was raised to 58% B within 15 min, then to 100% B within a further 15 min, followed by isocratic elution in 100% B for a further 10 min. The HPLC was coupled to a photodiode array detector for ultraviolet spectroscopic comparisons.[3]

- Ultraviolet-visible (UV/VIS) spectra were recorded using a Cary 4G Spectrophotometer using spectroscopic grade solvents. The data are displayed as λ_{max} wavenumber of the absorption peak in nm.

6.2 Experimental Procedures for Chapter Two

The Synthesis of 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (2.27)



2-Chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) was synthesised following a modified literature procedure.[4] To a stirred solution of 2-chloro-5-hydroxy-7-methoxy-1,4-naphthoquinone (**2.25**) (3.69g, 15mmol) in chloroform (20mL) was added silver(I) oxide (9.76g, 42mmol) and methyl iodide (4.70mL, 75mmol). The reaction mixture was stirred at room temperature for 48h, following which time it was filtered through celite, washed with additional chloroform and the filtrate concentrated *in vacuo*. The yellow powder was then purified by flash column chromatography (R_f 0.55, 50% ethyl acetate/ petroleum spirits) and isolated in 58% yield. The data obtained for naphthoquinone **2.27** was consistent with that reported in the literature.[4]

^{1}H NMR ($CDCl_3$): δ 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.73 (d, 1H, J = 2Hz, aromatic C*H*), 7.03 (s, 1H, quinone C*H*) and 7.29 (d, 1H, J = 2Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$): δ 56.1 (OCH_3), 56.5 (OCH_3), 104.6 (aromatic *C*H), 104.6 (aromatic *C*H), 113.8 (quaternary *C*Cl), 135.1 (quaternary *C*), 138.2 (quinone *C*H), 142.7 (quaternary *C*), 162.0 (quaternary aromatic *C*OMe), 164.8 (quaternary aromatic *C*OMe), 178.4 (carbonyl *C*), 180.7 (carbonyl *C*)

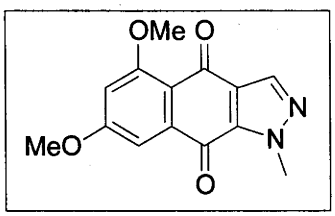
EIMS *m/z* 254 (58%, $C_{12}H_9{}^{37}ClO_4$), 252 (100%, $C_{12}H_9{}^{35}ClO_4$), 217 (98%, $M^{+\cdot}$-Cl), 189 (46%, $M^{+\cdot}$-Cl –CO)

HREIMS *m/z* $M^{+\cdot}$ 254.0160 (calcd for $C_{12}H_9{}^{37}ClO_4$ 254.0160)
m/z $M^{+\cdot}$ 252.0185 (calcd for $C_{12}H_9{}^{35}ClO_4$ 252.0189)

Melting Point 184°C (Lit. 187.5-188°C)[4]

Infrared ν_{max} (KBr): 2947 m, 1735 m, 1681 m, 1651 m, 1589 m, 1458 m, 1265 s, 1157 s, 1080 s, 840 w cm^{-1}

*The Synthesis of 5,7-dimethoxy-1-methyl-1H-benzo[f]indazole-4,9-dione (**2.30**)*



CAUTION: Diazomethane is potentially explosive and the use of ground-glass joints or boiling chips should be avoided. This reaction was carried out in a purpose-built distillation apparatus.[5]

To a solution of potassium hydroxide (0.505g, 9mmol) in water (1mL) was added 96% ethanol (2.5mL) and the solution warmed to 60-65°C. A solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (2.165g, 10mmol) in diethyl ether (25mL) was then added dropwise, followed by the addition of diethyl ether (30mL). The ethereal diazomethane solution thus generated was distilled into a solution of 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) (200 mg, 0.8mmol) in ether (10mL). The reaction vessel was stopped with a rubber bung and left to stand overnight at room temperature. The solvent was then allowed to evaporate. The resulting residue was purified by flash column chromatography (R_f 0.30, 50% ethyl acetate: petroleum spirits) and 5,7-dimethoxy-1-methyl-1H-benzo[f]indazole-4,9-dione (**2.30**) was isolated in 76% yield.

^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H, OC*H*$_3$), 3.96 (s, 3H, OC*H*$_3$), 4.26 (s, 3H, N-C*H*$_3$), 6.75 (d, 1H, *J* = 3Hz, aromatic C*H*), 7.37 (d, 1H, *J* = 3Hz, aromatic C*H*), 7.95 (s, 1H, vinylic C*H*)

^{13}C NMR ($CDCl_3$) δ 39.0 (N-*C*H$_3$), 56.0 (O*C*H$_3$), 56.5 (O*C*H$_3$), 104.3 (aromatic *C*H), 104.7 (aromatic *C*H), 115.5 (quaternary aromatic *C*), 125.0 (quaternary aromatic *C*), 136.1 (quaternary aromatic *C*), 137.1 (vinylic *C*H), 137.7 (quaternary aromatic *C*), 162.8 (aromatic *C*-OMe), 164.3 (aromatic *C*-OMe), 175.8 (carbonyl *C*), 178.6 (carbonyl *C*)

gHMBC (^{1}H-^{13}C correlations): 3.94-56.0 ($^1J_{CH}$), 3.96-56.5 ($^1J_{CH}$), 4.26-39.0 ($^1J_{CH}$), 6.75-104.3 ($^1J_{CH}$), 6.75-162.8 ($^2J_{CH}$), 6.75-164.3 ($^2J_{CH}$), 7.37-104.7 ($^1J_{CH}$), 7.37-164.3 ($^2J_{CH}$), 7.95-136.1 ($^1J_{CH}$)

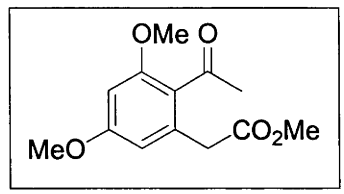
EIMS *m/z* 272 (100%, $M^{+\cdot}$), 243 (86%, $M^{+\cdot}$- NMe), 241 (52%), 225 (31%)

HREIMS *m/z* $M^{+\cdot}$ 272.0799 (calcd for $C_{14}H_{12}N_2O_4$ 272.0797)

Melting Point 201-202°C

Infrared ν_{max} (KBr): 3433 s, 1681 m, 1589 w, 1496 m, 1427 w, 1272 m, 1218 m, 1157 m, 1041 s, 802 w cm^{-1}

The Synthesis of Methyl 2-acetyl-3,5-dimethoxyphenylacetate (2.6)



Methyl 2-acetyl-3,5-dimethoxyphenylacetate (**2.6**) was synthesised via a modified literature procedure.[6] To a solution of methyl 3,5-dimethoxyphenylacetate (**2.34**) (4.4g, 21mmol) in acetic anhydride (50mL) was added 40% perchloric acid (0.1mL). The solution was stirred under nitrogen at room temperature for 1.5h, following which time the reaction mixture was poured onto ice-water and extracted with ethyl acetate (50mL). The organic layer was washed with 10% sodium hydrogen carbonate until effervescence ceased and then with brine (50mL). The organic layer was separated and dried with magnesium sulfate, filtered and concentrated *in vacuo* to give the ketone **2.6** as a crude product. The ketone **2.6** was purified by flash column chromatography (R_f 0.46, 15% ethyl acetate: petroleum spirits) to give methyl 2-acetyl-3,5-dimethoxyphenylacetate (**2.6**) in 84% yield. The data spectroscopic and physical data obtained was consistent with that reported in the literature.[6] The NMR data for ketone **2.6** has not been reported previously.

^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H, C(O)C*H*$_3$), 3.65 (s, 3H, CO_2CH_3), 3.67 (s, 2H, C*H*$_2$), 3.78 (s, 3H, OC*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 6.32 (d, 1H, J = 2Hz, aromatic C*H*), 6.38 (d, 1H, J = 2Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 32.2 (C(O)*C*H$_3$), 39.1 (*C*H$_2$), 51.9 (O*C*H$_3$), 55.4, (O*C*H$_3$), 55.6 (CO_2CH_3), 60.4 (*C*O$_2$Me), 97.4 (aromatic *C*H), 108.2 (aromatic *C*H), 123.5 (quaternary aromatic *C*), 134.9 (quaternary aromatic *C*), 159.4 (quaternary aromatic *C*), 161.5 (quaternary aromatic *C*), 171.7 (carbonyl *C*)

EIMS *m/z* 252 (73%, $M^{+\cdot}$), 237 (63%, $M^{+\cdot}$- Me), 221 (32%), 210 (62%), 209 (100%, $M^{+\cdot}$- Me- CO), 192 (64%)

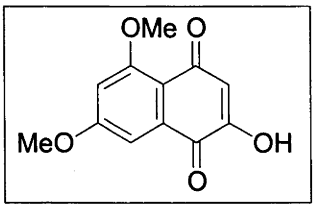
HREIMS *m/z* $M^{+\cdot}$ 252.1000 (calcd for $C_{13}H_{16}O_5$ 252.0998)

Anal. Calc. for $C_{13}H_{16}O_5$: C, 61.90, H, 6.39. Found: C, 61.95, H, 6.24%.

Melting Point 59-61°C (Lit. 60-61°C)[6]

Infrared ν_{max} (KBr): 3433 w, 2947 w, 1720 m, 1666 m, 1596 s, 1427 w, 1319 m, 1280 m, 1203 s, 1164 s, 1095 w, 1018 w cm^{-1}

The Synthesis of 2-hydroxy-5,7-dimethoxy-1,4-naphthoquinone (2.7)



2-Hydroxy-5,7-dimethoxy-1,4-naphthoquinone (**2.7**) was synthesised following a modified literature procedure.[6] A solution of sodium ethoxide was freshly prepared by the addition sodium (0.3g, 13mmol) to dry ethanol (30mL). The solution was heated to reflux for 0.5h following the addition of methyl 2-acetyl-3,5-dimethoxyphenylacetate (**2.6**) (1.5g, 5.9mmol) in ethanol (20mL). The reaction mixture was then cooled and air was bubbled through the solution for 16h. The solvent was removed *in vacuo* following which 0.5 M sulfuric acid (200mL) was added to the resulting residue. The precipitate formed was then filtered, washed with water and dried to give the desired product as a yellow microcrystalline powder in 70% yield. The product was purified by recrystallisation from ethanol. The data obtained for naphthoquinone **2.7** was consistent with that reported in the literature.[6] The NMR data for naphthoquinone **2.7** has not been previously reported.

^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.18 (s, 1H, quinone C*H*), 6.77 (d, 1H, J = 2Hz, aromatic C*H*), 6.96 (s, 1H, O*H*), 7.26 (d, 1H, J = 2Hz, aromatic C*H*)

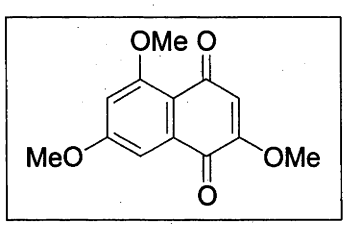
^{13}C NMR ($CDCl_3$) δ 55.3 (OCH_3), 56.2 (OCH_3), 103.5 (aromatic *C*H), 104.8 (aromatic *C*H), 112.8 (quinone *C*H), 133.3 (quaternary aromatic *C*), 154.8 (quaternary aromatic *C*), 161.2 (quaternary aromatic *C*), 163.6 (quaternary aromatic *C*), 170.4 (quaternary aromatic *C*), 181.8 (carbonyl *C*), 184.0 (carbonyl *C*)

EIMS *m/z* 234 (100%, $M^{+\cdot}$), 205 (37%, $M^{+\cdot}$-CO), 176 (25%), 135 (52%), 84 (57%)

HREIMS *m/z* $M^{+\cdot}$ 234.0531 (calcd for $C_{12}H_{10}O_5$ 234.0528)

Melting Point 215-216°C decomp. (Lit. 218-219°C decomp.)[6]

The Synthesis of 2,5,7-trimethoxy-1,4-naphthoquinone (2.1)



Method A[7]

1,1-Dimethoxyethene (**2.8**) (0.640g, 7.3mmol) was added to a stirred paste of 2-chloro-5-methoxy-1,4-benzoquinone (**2.9**) (0.25g, 1.4mmol) and glacial acetic acid (0.20mL) at room temperature. After the

exothermic reaction had subsided the mixture was refluxed for 3h, following which the solvent was evaporated under reduced pressure. The crude product mixture was then subjected to flash column chromatography (50% ethyl acetate/ petroleum spirits) to give the desired product in 11% yield.

Method B

To a solution of 2-chloro-5,7-dimethoxy-1,4-naphthoquinone (**2.27**) (0.1g, 0.40mmol) in dry methanol (60mL) was added potassium carbonate (111 mg, 0.80 mmol). The reaction mixture was heated gently at 60°C for 0.5h, following which time the reaction mixture was cooled, then concentrated *in vacuo*. The resulting dark residue was partitioned between water (100mL) and dichloromethane (100mL). The aqueous fraction was extracted repeatedly with dichloromethane until colour was no longer evident in the organic layer. The organic fractions were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give a deep red powder in 84% yield.

Method C

To a stirred solution of 2-hydroxy-5,7-dimethoxy-1,4-naphthoquinone (**2.7**) (0.20g, 0.9mmol) in chloroform (10mL) was added freshly purified silver(I) oxide (0.65g, 2.8mmol) and methyl iodide (0.9mL, excess). The reaction mixture was stirred at room temperature for 16h, following which the suspension was filtered through celite. The filtrate was concentrated *in vacuo* and the resulting residue was subjected to flash column chromatography (50% ethyl acetate/ petroleum spirits) to give the desired product in 80% yield. The data obtained for naphthoquinone **2.1** was consistent with that reported in the literature.[4]

R_f	0.66 (50% ethyl acetate/ petroleum spirits)
^{1}H NMR	($CDCl_3$): δ 3.82 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.00 (s, 1H, quinone C*H*), 6.73 (d, 1H, J = 2Hz, aromatic C*H*), 7.28 (d, 1H, J = 2Hz, aromatic C*H*)
^{13}C NMR	($CDCl_3$): δ 56.0 (OCH_3), 56.2 (OCH_3), 56.5 (OCH_3), 103.5 (aromatic *C*H), 105.0 (aromatic *C*H), 112.1 (quinone *C*H), 135.0 (quaternary *C*),

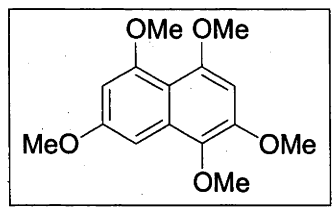
135.0 (quaternary *C*), 158.1 (*C*OMe), 161.5 (aromatic *C*OMe), 164.2 (aromatic *C*OMe), 180.5 (carbonyl *C*), 183.8 (carbonyl *C*)

EIMS *m/z* 248.00 (100%, $M^{+\cdot}$), 233 (7%, $M^{+\cdot}$ – Me), 219 (22%), 188 (18%), 177 (23%), 149 (35%), 119 (33%)

HREIMS *m/z* $M^{+\cdot}$ 248.0685 (calcd for $C_{13}H_{12}O_5$ 248.0685)

Melting Point 247-249°C (Lit. 250°C decomp.)[4]

The Synthesis of 1,2,4,5,7-pentamethoxynaphthalene (2.35)



To a stirred solution of 2,5,7-trimethoxy-1,4-naphthoquinone (**2.1**) (0.5g, 2.01mmol) in THF (50mL) at 0°C was added tetrabutylammonium bromide (1.7g, 5.27mmol). This was followed by the addition of aqueous sodium dithionite (100mL, 30% w/v), dimethyl sulfate (23.8mL, 0.25mol) and, finally, the slow addition of aqueous potassium hydroxide (150mL, 26% w/v) over 5 min. The reaction mixture was stirred at room temperature for 16h, following which ethyl acetate (100mL) was added. The aqueous layer was separated using a separatory funnel and then extracted with ethyl acetate (2 x 100mL). The organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give the naphthalene as the crude product. This was purified by flash column chromatography on SiO_2 using 25% ethyl acetate/ petroleum spirits (R_f 0.3) to give the product as a white solid (81%).

^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3H, OCH_3), 3.90 (s, 9H, 3 x OCH_3), 3.96 (s, 3H, OCH_3), 6.34 (d, 1H, J = 3Hz, aromatic C*H*), 6.47 (s, 1H, aromatic C*H*), 6.94 (d, 1H, J = 3Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 55.3 (OCH_3), 56.1 (OCH_3), 56.6 (OCH_3), 57.0 (OCH_3), 60.6 (OCH_3), 91.8 (aromatic *C*H), 94.7 (aromatic *C*H), 97.0 (aromatic *C*H), 108.9 (quaternary aromatic *C*), 132.9 (quaternary aromatic *C*), 136.0 (quaternary aromatic *C*), 149.09 (aromatic *C*OCH_3), 154.28 (aromatic *C*OCH_3), 158.51 (aromatic *C*OCH_3), 158.78 (aromatic *C*OCH_3)

EIMS *m/z* 278.1 (56%, $M^{+\cdot}$), 263.1 (100%, $M^{+\cdot}$–Me), 235 (37%), 220 (11%), 192 (13%), 148 (22%)

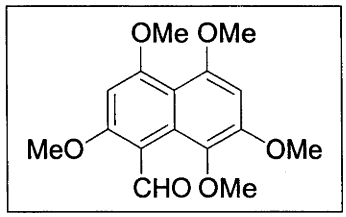
HREIMS *m/z* $M^{+\cdot}$ 278.1150 (calcd for $C_{15}H_{18}O_5$ 278.1154)

Anal. Calc. for $C_{15}H_{18}O_5$: C, 64.74, H, 6.52. Found: C, 64.34, H, 6.54%.

Melting Point 118°C

Infrared ν_{max} (KBr): 2924 w, 2839 w, 2360 m, 1620 s, 1473 m, 1350 s, 1265 m, 1211 m, 1126 m, 1041 s, 817 m cm^{-1}

The Synthesis of 8-formyl-1,2,4,5,7-pentahydroxynaphthalene (2.36)



N, N- Dimethylformamide (7.23mmol, 0.56mL) was added dropwise to a stirred solution of freshly distilled phosphoryl chloride (9.44mmol, 0.88mL) in dry dichloromethane (40mL) at 0°C. The solution was stirred for 0.5h at 0°C, after which time 1,2,4,5,7-pentamethoxynaphthalene (**2.35**) (0.5g, 1.80mmol) was added and the reaction mixture was stirred for a further 0.5h at 0°C. The reaction mixture was allowed to warm to room temperature and stirring continued for a further 16h. The reaction was then quenched by the addition of aqueous sodium acetate (50mL, 10% w/v). The aqueous layer was extracted exhaustively with ethyl acetate (3 x 50mL) and the combined organic fractions were dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give the aldehyde **2.36** as the crude product. The aldehyde **2.36** was purified by flash column chromatography on SiO_2 using 80% ethyl acetate/ petroleum spirits (R_f 0.50) to give the product as a white microcrystalline powder (83%).

^{1}H NMR ($CDCl_3$) δ 3.55 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.91 (s, 6H, 2 x OCH_3), 6.36 (s, 1H, aromatic C*H*), 6.47 (s, 1H, aromatic C*H*), 10.41 (s, 1H, C*H*O)

^{13}C NMR ($CDCl_3$) δ 56.1 (OCH_3), 56.3 (2 x OCH_3), 56.7 (OCH_3), 59.8 (OCH_3), 91.7 (aromatic *C*H), 94.6 (aromatic *C*H), 108.3 (quaternary aromatic *C*), 112.2 (quaternary aromatic *C*), 130.8 (quaternary aromatic *C*), 135.5 (quaternary aromatic *C*), 150.4 (quaternary aromatic *C*OMe), 154.9 (quaternary aromatic *C*OMe), 156.4 (quaternary aromatic *C*OMe), 160.8 (quaternary aromatic *C*OMe), 192.4 (1s, *C*HO)

EIMS *m/z* 306.1 (85%, $M^{+\cdot}$), 291.1 (93%, $M^{+\cdot}$ – Me), 275.1 (100%, $M^{+\cdot}$ – CHO), 263 (27%), 248 (19%)

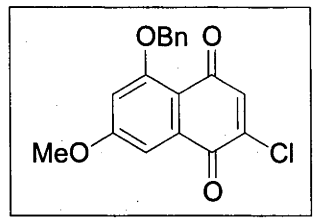
HREIMS *m/z* $M^{+\cdot}$ 306.1099 (calcd for $C_{16}H_{18}O_6$ 306.1103)

Anal. Calcd for $C_{16}H_{18}O_6$: C, 62.74, H, 5.92. Found: C, 63.02, H, 6.04%.

Melting Point 158.5°C

Infrared ν_{max} (KBr): 2932 w, 2839 w, 1681 s, 1589 s, 1512 w, 1458 s, 1396 m, 1334 s, 1272 m, 1211 s, 1041 s cm^{-1}

*The Synthesis of 2-chloro-5-benzyloxy-7-methoxy-1,4-naphthoquinone (**2.46**)*



To a solution of 2-chloro-5-hydroxy-7-methoxy-1,4-naphthoquinone (**2.25**) (0.85g, 3.6mmol) in chloroform (10mL) was added benzyl bromide (0.85mL, excess) and silver(I) oxide (3.3g, 14.2mmol) and the reaction mixture was stirred at room temperature for 96h. The suspension was filtered through celite and the solvent concentrated *in vacuo* to give the crude product mixture, which was then purified by column chromatography (R_f 0.42, 15% ethyl acetate/ petroleum spirits) to give 2-chloro-5-benzyloxy-7-methoxy-1,4-naphthoquinone (**2.46**) in 54% yield.

^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, OCH_3), 5.19 (s, 2H, OCH_2Ph), 6.74 (s, 1H, vinyl C*H*) 7.01 (s, 1H, aromatic C*H*), 7.22-7.41 (m, 4H, aromatic CH), 7.51-7.54 (m, 2H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 56.0 (OCH_3), 70.8 (OCH_2Ph), 105.0 (aromatic *C*H), 105.9 (aromatic *C*H), 114.0 (quaternary aromatic *C*), 126.5 (aromatic *C*H), 127.9 (2 x aromatic *C*H), 128.6 (2 x aromatic *C*H), 135.0 (quaternary aromatic *C*), 135.6 (quaternary aromatic *C*), 138.0 (quinone *C*H), 142.6 (quinone *C*Cl), 160.8 (quaternary aromatic *C*), 164.5 (quaternary aromatic *C*), 178.3 (carbonyl *C*), 180.4 (carbonyl *C*)

EIMS *m/z* 330 (15%, $M^{+\cdot}$ $C_{18}H_{13}O_4{}^{37}Cl$), 328 (33%, $M^{+\cdot}$ $C_{18}H_{13}O_4{}^{35}Cl$), 272 (6%), 237 (7%), 222 (11%), 91 (100%)

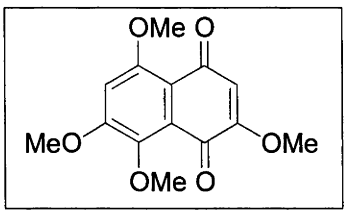
HREIMS *m/z* $M^{+\cdot}$ 330.0473 (calcd for $C_{18}H_{13}O_4{}^{37}Cl$ 330.0473)
m/z $M^{+\cdot}$ 328.0500 (calcd for $C_{18}H_{13}O_4{}^{35}Cl$ 328.0502)

Anal. Calcd for $C_{18}H_{13}ClO_4$: C, 65.76, H, 3.99. Found: C, 65.33, H, 4.11%.

Melting Point 121.5-123.5°C

Infrared ν_{max} (KBr): 3418 w, 1682 s, 1645 s, 1593 s, 1556 w, 1454 w, 1438 w, 1385 w, 1328 s, 1302 m, 1294 m, 1276 s, 1259 s, 1233 m, 1203 m, 1170 m, 1141 m, 1090 w, 1027 w cm^{-1}

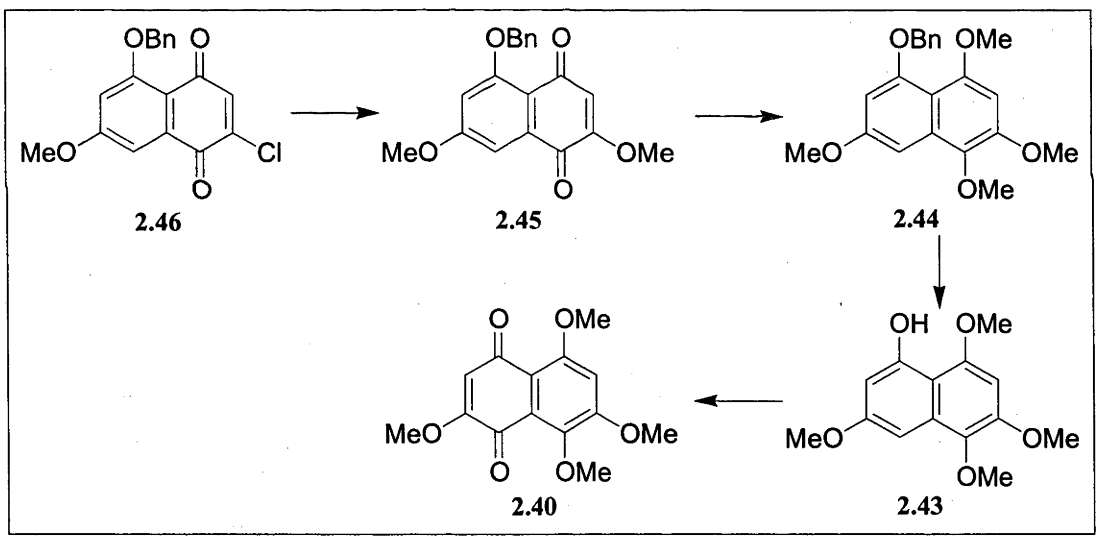
*The Synthesis of 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**)*



Method A

To a stirred solution of 8-formyl-1,2,4,5,7-pentahydroxynaphthalene (**2.36**) (0.2g, 0.65mmol) in methanol (35mL) was added 30% aqueous hydrogen peroxide (1mL) and concentrated sulfuric acid (6.5 μL). The reaction mixture was stirred at room temperature for 1.5h and then poured onto cold, aqueous sodium bicarbonate (50mL, 10% w/v) and immediately extracted with ethyl acetate (3 x 50mL). The combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give the deep red naphthoquinone **2.40**. The compound was then recrystallised from ethyl acetate/ petroleum spirits to give red needles in 72% yield.

Method B



To a solution of 2-chloro-5-benzyloxy-7-methoxy-1,4-naphthoquinone (**2.46**) (0.2g, 0.62mmol) in dry methanol (65mL) was added anhydrous potassium carbonate (0.174g, 1.26mmol). The reaction mixture was stirred at room temperature for 0.5h to give 2,7-dimethoxy-5-benzyloxy-1,4-naphthoquinone (**2.45**) in 93% yield following purification (R_f 0.60, 25% ethyl acetate/ petroleum spirits) via flash column chromatography.

Data obtained for naphthoquinone **2.45**:

^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.23 (s, 2H, CH_2Ph), 6.01 (s, 1H, quinone C*H*), 6.75 (d, 1H, J = 0.5Hz, aromatic C*H*), 7.21-7.58 (m, 6H, 6 x aromatic C*H*)

EIMS *m/z* 324 (32%, $M^{+\cdot}$), 235 (9%, $M^{+\cdot}$ -OBn), 190 (5%), 188 (8%), 149 (3%), 91 (100%)

HRMS *m/z* $M^{+\cdot}$ 324.1000 (calcd for $C_{19}H_{16}O_5$ 324.0998)

Melting Point 128-130°C

Infrared ν_{max} (KBr): 3419 w, 2937 w, 2842 w, 1698 m, 1682 m, 1651 m, 1644 m, 1622 m, 1593 s, 1455 m, 1440 w, 1384 w, 1312 s, 1259 s, 1239 m, 1164 m, 1022 m cm^{-1}

To a stirred solution of 2,7-dimethoxy-5-benzyloxy-1,4-naphthoquinone **(2.45)** (0.187g, 0.58mmol) in THF (20mL) at 0°C was added tetrabutylammonium bromide (0.490g, 1.52mmol), followed by the addition of sodium dithionite (29mL, 30% w/v), dimethyl sulfate (6.8mL, 71mmol) and, finally, the slow addition of aqueous potassium hydroxide (45mL, 26% w/v) over 5 min. The reaction mixture was stirred for 16h at room temperature, following which ethyl acetate was added (50mL). The aqueous layer was separated using a separatory funnel and extracted with ethyl acetate (2 x 50mL). The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give the naphthalene **2.44** as the crude product. This was purified by flash column chromatography (R_f 0.32, 15% ethyl acetate/ petroleum spirits) and the product was isolated as a colourless oil (56%).

Data obtained for naphthalene **2.44**:

^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2Ph), 6.49 (d, 1H, J = 0.5Hz, aromatic C*H*), 6.55 (s, 1H, aromatic C*H*), 7.02 (d, 1H, J = 0.5Hz, aromatic C*H*), 7.27-7.62 (m, 5H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 55.2 (OCH_3), 56.6 (OCH_3), 56.8 (OCH_3), 60.6 (OCH_3), 70.8 (OCH_2Ph), 92.2 (aromatic *C*H), 94.6 (aromatic *C*H), 98.7 (aromatic *C*H), 109.3 (quaternary *C*), 126.8 (2 x aromatic *C*H), 127.5 (aromatic *C*H), 128.3 (2 x aromatic *C*H), 132.8 (quaternary *C*), 135.9 (quaternary *C*),

137.2 (quaternary *C*), 149.1 (quaternary *C*), 154.4 (quaternary *C*), 157.4 (quaternary *C*), 158.7 (quaternary *C*)

EIMS *m/z* 354 (100%, $M^{+\cdot}$), 339 (31%, $M^{+\cdot}$-Me), 263 (54%, $M^{+\cdot}$-Bn), 235 (16%), 220 (26%), 204 (29%), 91 (96%)

HREIMS *m/z* $M^{+\cdot}$ 354.1465 (calcd for $C_{21}H_{22}O_5$ 354.1467)

Melting Point 71.5-72.5°C

Infrared ν_{max} (KBr): 2996 w, 2936 w, 1621 s, 1615 s, 1591 s, 1463 m, 1455 m, 1408 m, 1394 m, 1390 m, 1351 s, 1266 m, 1242 m, 1200 m, 1157 s, 1124 m, 1066 s, 1044 s, 847 w cm^{-1}

To a solution of 1,2,4,7-tetramethoxy-5-benzyloxynaphthalene (**2.44**) (115 mg, 0.33mmol) in ethyl acetate (20mL) was added 10% palladium on carbon (12mg) and the reaction mixture stirred at room temperature under a hydrogen atmosphere for 96h. The resultant suspension was then poured through celite and the volume of the filtrate concentrated *in vacuo* to give a mixture of compounds. The mixture was separated chromatographically (15% ethyl acetate/ petroleum spirits) to give the desired naphthol **2.43** as the major product (30%).

Data obtained for naphthol **2.43**:

R_f 0.19 (15% ethyl acetate/ petroleum spirits)

^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.96 (s, 3H, OC*H*$_3$), 4.01 (s, 3H, OC*H*$_3$), 6.41 (d, 1H, *J* = 2Hz, aromatic C*H*), 6.43 (s, 1H, aromatic C*H)*, 6.88 (d, 1H, *J* = 2Hz, aromatic C*H*), 9.21 (s, 1H, O*H*)

^{13}C NMR ($CDCl_3$) δ 55.3 (O*C*H$_3$), 56.3 (O*C*H$_3$), 57.1 (O*C*H$_3$), 60.6 (O*C*H$_3$), 91.9 (aromatic *C*H), 92.8 (aromatic *C*H), 100.2 (aromatic *C*H), 106.9 (quaternary *C*), 132.5 (quaternary *C*), 136.9 (quaternary *C*), 148.6 (quaternary *C*-OMe), 153.1 (quaternary *C*-OMe), 155.9 (quaternary *C*-OMe), 159.8 (quaternary *C*-OMe)

EIMS *m/z* 264 (60%, $M^{+\cdot}$), 249 (100%, $M^{+\cdot}$–Me), 235 (14%), 221 (49%, $M^{+\cdot}$–Me –CO), 206 (29%), 175 (11%), 135 (15%)

HREIMS *m/z* $M^{+\cdot}$ 264.1001 (cald for $C_{14}H_{16}O_5$ 264.0998)

Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63, H, 6.10. Found: C, 63.64, H, 6.00%.

Melting Point 115-116°C

Infrared ν_{max} (KBr): 3384 s, 2935 m, 2845 m, 1634 s, 1618 m, 1592 w, 1452 m, 1384 s, 1354 m, 1274 m, 1229 w, 1150 s, 1122 m, 1109 m, 1043 m, 1017 w cm^{-1}

To a solution of 5-hydroxy-1,2,4,7-tetramethoxynaphthalene (**2.43**) (26mg, 0.1mmol) in acetonitrile (2mL) was added potassium nitrosodisulfonate (30mg, 0.11mmol) and potassium dihydrogen phosphate (15mg, 0.11mmol) in water (0.5mL) with sonication. The reaction mixture was then stirred for 16h at room temperature without further sonication. The solvent was concentrated *in vacuo* to give the quinone **2.40**. The compound was then purified by flash column chromatography (R_f 0.12, 80% ethyl acetate/ petroleum spirits) to give the pure naphthoquinone **2.40** in quantitative yield. The spectroscopic data obtained for naphthoquinone **2.40** was consistent with that reported previously.[8,9]

Data obtained for naphthoquinone **2.40**:

^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$), 3.92 (s, 3H, OC*H*$_3$), 3.93 (s, 3H, OC*H*$_3$), 5.92 (s, 1H, quinone C*H*), 6.72 (s, 1H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 56.1 (O*C*H$_3$), 56.2 (O*C*H$_3$), 56.7 (O*C*H$_3$), 61.2 (O*C*H$_3$), 102.1 (quinone *C*H), 110.8 (aromatic *C*H), 112.5 (quaternary *C*), 125.6 (quaternary *C*), 144.1 (quaternary *C*), 157.4 (quaternary *C*-OMe), 158.7 (quaternary *C*-OMe), 159.1 (quaternary *C*-OMe), 179.6 (carbonyl *C*), 183.6 (carbonyl *C*)

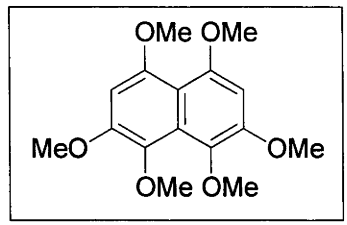
EIMS *m/z* 280 (22%, $M^{+\cdot}$+2H), 278.0 (100%, $M^{+\cdot}$), 265 (31%), 263 (17%, $M^{+\cdot}$-Me), 217 (24%), 207 (16%), 189 (11%), 149 (12%)

HREIMS *m/z* $M^{+\cdot}$ 278.0785 (calcd for $C_{14}H_{14}O_6$ 278.0790)

Melting Point 162 °C (Lit. 169-171°C)

Infrared ν_{max} (KBr): 3418 w, 2946 w, 2848 w, 1679 m, 1644 s, 1627 s, 1578 w, 1548 m, 1471 m, 1353 m, 1313 m, 1259 s, 1233 s, 1216 s, 1177 m, 1094 m, 1034 s, 845 m cm^{-1}

*The Synthesis of 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**)*



To a stirred solution of 2,5,7,8-tetramethoxy-1,4-naphthoquinone (**2.40**) (0.18g, 0.65mmol) in THF (20mL) at 0°C was added tetrabutylammonium bromide (0.55g, 1.7mmol), followed by the addition of aqueous sodium dithionite (32mL, 30% w/v), dimethyl sulfate (7.66mL, 80mmol) and, finally, the slow addition of aqueous potassium hydroxide (50mL, 26% w/v) over 5 min. The reaction mixture was stirred for 16h at room temperature, following which ethyl acetate (100mL) was added. The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 50mL). The organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give the naphthalene **2.39** which was purified by flash column chromatography on SiO_2 using 50% ethyl acetate/ petroleum spirits (R_f 0.51). The product was isolated as a white powder in 74% yield.

^{1}H NMR ($CDCl_3$) δ 3.79 (s, 6H, 2 x OC*H*$_3$), 3.91 (s, 6H, 2 x OC*H*$_3$), 3.95 (s, 6H, 2 x OC*H*$_3$), 6.54 (s, 2H, 2 x aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 56.5 (2 x OCH_3), 57.2 (2 x OCH_3), 61.9 (2 x OCH_3), 95.0 (2 x aromatic *C*H), 110.2 (quaternary aromatic *C*), 126.9 (quaternary aromatic *C*), 135.9 (2 x aromatic *C*-OMe), 150.3 (2 x aromatic *C*-OMe), 154.3 (2 x aromatic *C*-OMe)

EIMS *m/z* 308 (100%, $M^{+\cdot}$), 293 (80%, $M^{+\cdot}$–Me), 278 (13%), 265 (20%), 261 (45%), 233 (24%)

HREIMS *m/z* $M^{+\cdot}$ 308.1257 (calcd for $C_{16}H_{20}O_6$ 308.1260)

Melting Point 82 °C

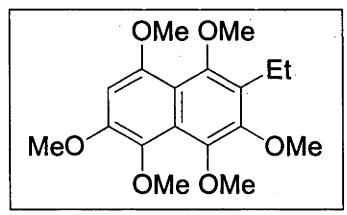
Infrared ν_{max} (KBr): 3443 w, 2936 m, 2839 m, 1735 w, 1719 w, 1601 m, 1454 m, 1438 m, 1400 w, 1361 m, 1331 s, 1261 m, 1209 s, 1170 w, 1047 s, 999 w cm^{-1}

*The Synthesis of 3-ethyl-1,2,4,5,7,8-hexamethoxynaphthalene (**2.47**) and 3,6-diethyl-1,2,4,5,7,8-hexamethoxynaphthalene (**2.50**)*

To a solution of 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) (0.1g, 0.32mmol) in dry THF (2mL) in a two-necked 10 mL round-bottomed flask was added *n*-butyl lithium

(0.21mL, 0.38mmol) under nitrogen at -78°C with stirring. TMEDA (0.06mL, 0.38mmol) was added to the reaction mixture and the solution stirred for 1h at -78°C before it was warmed to room temperature. The reaction mixture was then cooled to -78°C and ethyl iodide (0.1mL, excess) was added and the solution stirred for 1h at -78°C followed by warming to room temperature. The reaction mixture was quenched with water and extracted with ethyl acetate (3 x 10mL). The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the crude monoethylated naphthalene **2.47**, diethylated naphthalene **2.50** and starting material **2.39** in a 5: 4: 1 ratio. The compounds were then separated using flash column chromatography with 15% ethyl acetate/petroleum spirits (R_f 0.28, 0.81 and 0.1 respectively).

Data obtained for naphthalene **2.47**:



^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7Hz, CH_2CH_3), 2.77 (q, 2H, J = 7Hz, CH_2CH_3), 3.75 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.62 (s, 1H, aromatic C*H*)

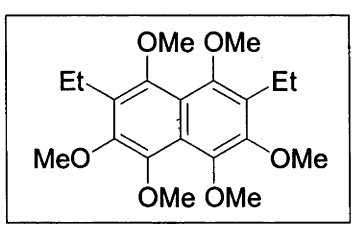
^{13}C NMR ($CDCl_3$) δ 15.5 (CH_2CH_3), 17.9 (CH_2CH_3), 56.7 (OCH_3), 56.9 (OCH_3), 61.1 (OCH_3), 61.6 (OCH_3), 61.8 (OCH_3), 62.5 (OCH_3), 95.9 (aromatic *C*H), 114.4 (quaternary aromatic *C*), 125.1 (quaternary aromatic *C*), 128.4 (quaternary aromatic *C*), 136.6 (quaternary aromatic *C*), 143.3 (quaternary aromatic *C*), 149.2 (quaternary aromatic *C*), 150.3 (quaternary aromatic *C*), 150.9 (quaternary aromatic *C*), 152.7 (quaternary aromatic *C*)

EIMS *m/z* 336 (100%, $M^{+\cdot}$), 321 (83%, $M^{+\cdot}$-Me), 306 (21%, $M^{+\cdot}$-Et), 289 (40%), 275 (12%), 263 (27%), 233 (11%)

HREIMS *m/z* $M^{+\cdot}$ 336.1570 (calcd for $C_{18}H_{24}O_6$ 336.1573)

Melting Point 45 °C

Data obtained for naphthalene **2.50**:



^{1}H NMR ($CDCl_3$) δ 1.24 (t, 6H, J = 7Hz, CH_2CH_3), 2.81 (q, 4H, J = 7Hz, CH_2CH_3), 3.76 (s,

6H, 2 x OCH_3), 3.89 (s, 6H, 2 x OCH_3), 3.98 (s, 6H, 2 x OCH_3)

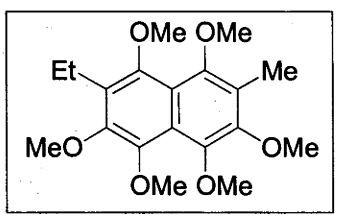
^{13}C NMR ($CDCl_3$) δ 15.6 (2 x CH_2CH_3), 18.0 (2 x CH_2CH_3), 61.1 (2 x OCH_3), 61.4 (2 x OCH_3), 62.63 (2 x OCH_3), 118.3 (quaternary aromatic *C*), 123.4 (quaternary aromatic *C*), 129.7 (2 x quaternary aromatic *C*), 143.9 (2 x quaternary aromatic *C*), 149.0 (2 x quaternary aromatic *C*), 149.7 (2 x quaternary aromatic *C*)

EIMS *m/z* 364 (100%, $M^{+\cdot}$), 349 (66%, $M^{+\cdot}$-Me), 334 (19%, $M^{+\cdot}$-Et), 321 (35%), 291 (20%), 182 (29%)

HREIMS *m/z* $M^{+\cdot}$ 364.1885 (calcd for $C_{20}H_{28}O_6$ 364.1886)

Melting Point 52-53°C

*The Synthesis of 3-ethyl-1,2,4,5,7,8-hexamethoxy-6-methylnaphthalene (**2.48**)*



To a stirred solution of 3-ethyl-1,2,4,5,7,8-hexamethoxynaphthalene (**2.47**) (51mg, 0.15mmol) in dry THF (1mL) was added *n*-butyl lithium (0.1mL, 0.18mmol) at -78°C. TMEDA (0.03mL, 0.18mmol) was added to the reaction mixture and the solution stirred for 1h at -78°C prior to warming to room temperature. The reaction mixture was then cooled to -78°C and methyl iodide (0.05mL, excess) was added and the solution was stirred for 1h at -78°C followed by warming to room temperature. The reaction mixture was quenched with water and extracted with diethyl ether (3 x 5mL). The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the crude naphthalene **2.48**, which was purified by flash column chromatography (R_f 0.64, 15% ethyl acetate/ petroleum spirits) to give a white solid in 95% yield.

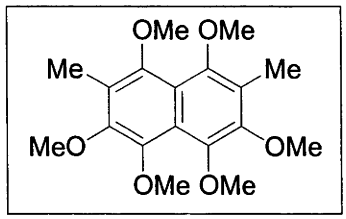
^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, *J* = 6Hz, CH_2CH_3), 2.32 (s, 3H, CH_3), 2.78 (q, 2H, *J* = 6Hz, CH_2CH_3), 3.72 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3)

EIMS *m/z* 350.0 (100%, $M^{+\cdot}$), 335.0 (65%, $M^{+\cdot}$-Me), 320 (14%), 307 (29%), 277 (16%), 175 (17%)

HREIMS *m/z* $M^{+\cdot}$ 350.1734 (calcd for $C_{19}H_{26}O_6$ 350.1729)

Melting Point 71-72 °C

The Synthesis of 3,6-dimethyl-1,2,4,5,7,8-hexamethoxynaphthalene (2.49)



To a stirred solution of 1,2,4,5,7,8-hexamethoxynaphthalene (**2.39**) (0.10g, 0.30mmol) in dry THF (2mL) was added *n*-butyl lithium (0.44mL, 1.20mmol) at -78°C. TMEDA (0.12mL, 0.72mmol) was added to the reaction mixture and the solution stirred for 1h at -78°C before it was warmed to room temperature. The reaction mixture was then cooled to -78°C and methyl iodide (0.20mL, excess) was added and the solution was stirred for 1h at -78°C followed by warming to room temperature. The reaction mixture was quenched with water and extracted with diethyl ether several times. The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the naphthalene **2.49**. The naphthalene **2.49** was purified by flash column chromatography to give a white solid (R_f 0.67, 15% ethyl acetate/ petroleum spirits) in quantitative yield.

^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H, 2 x CH_3), 3.73 (s, 6H, 2 x OCH_3), 3.87 (s, 6H, 2 x OCH_3), 3.91 (s, 6H, 2 x OCH_3)

^{13}C NMR ($CDCl_3$) δ 9.5 (2 x aromatic CH_3), 60.6 (2 x OCH_3), 61.6 (2 x OCH_3), 61.6 (2 x OCH_3), 123.6 (2 x quaternary aromatic *C*), 144.0 (2 x quaternary aromatic *C*), 149.2 (2 x quaternary aromatic *C*), 149.7 (2 x quaternary aromatic *C*)

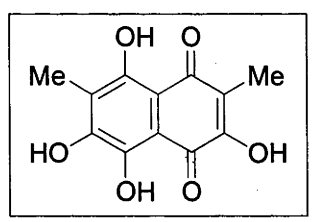
(N. B. Two quaternary aromatic signals were not evident in the ^{13}C spectrum obtained.)

EIMS *m/z* 336 (100%, $M^{+\cdot}$), 321 (54%, $M^{+\cdot}$-Me), 306 (17%), 293 (37%), 263 (21%), 168 (24%)

HREIMS *m/z* $M^{+\cdot}$ 336.1573 (calcd for $C_{18}H_{24}O_6$ 336.1573)

Melting Point 97-98°C

The Synthesis of 2,5,7,8-tetrahydroxy-3,6-dimethyl-1,4-naphthoquinone (Aureoquinone, 1.14)



To a stirred solution of naphthalene **2.49** (75mg, 0.30mmol) in dry dichloromethane (1mL) was added boron tribromide (1M in dichloromethane, 1.8mL) at -78°C. The reaction mixture was warmed to room

temperature and stirred for a further 48h after which time water (2mL) was added prior to the addition of ethyl acetate (5mL). The organic layer was removed using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 5mL). The red organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the deep red-purple solid in quantitative yield. The spectroscopic data obtained for naphthazarin **1.14** was consistent with that reported previously.[10]

R_f 0.26 (1:1 MeOH:$CHCl_3$)

^{1}H NMR ($CDCl_3$) δ 2.14 (s, 6H, C*H*$_3$), 13.44 (s, 1H, O*H*)

EIMS *m/z* 250 (100%, $M^{+\cdot}$), 235 (5%), 222 (31%, $M^{+\cdot}$-CO), 204 (13%), 176 (17%)

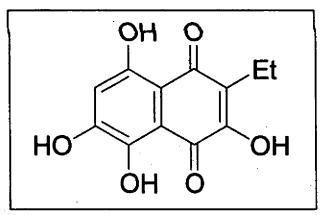
HREIMS *m/z* $M^{+\cdot}$ 250.0478 (calcd for $C_{12}H_{10}O_6$ 250.0477)

Melting Point 273°C (Lit. 268-9°C)[10]

UV/Vis (MeOH): 233, 327 nm

(H_2O): 273, 297, 341 nm

The Synthesis of 3-ethyl-2,5,7,8-tetrahydroxy-1,4-naphthoquinone (1.15)



To a stirred solution of naphthalene **2.47** (7.50mg, 0.03mmol) in dry dichloromethane (0.5mL) was added boron tribromide (1M in dichloromethane, 0.18mL) at -78 °C. The reaction mixture was warmed to room temperature and stirred for a further 48h, after which time water (2mL) was added prior to the addition of ethyl acetate (5mL). The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 5mL). The red organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the deep red-purple solid in quantitative yield. The spectroscopic data obtained for naphthazarin **1.15** was consistent with that reported previously.[11]

^{1}H NMR ($CDCl_3$) δ 1.10 (t, 3H, *J* = 7Hz, CH_2CH_3), 2.61 (q, 2H, *J* = 7Hz, CH_2CH_3)

N. B. O*H* signals not observed

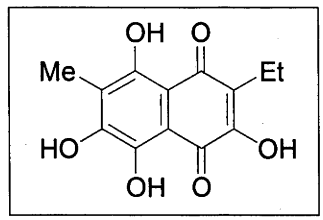
EIMS *m/z* 250 (100%, $M^{+\cdot}$), 234 (72%, $M^{+\cdot}$-Me-H), 207 (40%), 191 (29%), 137 (22%)

HREIMS *m/z* $M^{+\cdot}$ 250.0476 (calcd for $C_{12}H_{10}O_6$ 250.0477)

Melting Point 192°C (Lit. 183-186°C)[11]

UV/Vis (MeOH): 228, 263, 315, 483, 515, 552 nm

*The Synthesis of 3(6)-ethyl-2,5,7,8-tetrahydroxy-6(3)-methyl-1,4-naphthoquinone (Boryquinone, **1.16**)*



To a stirred solution of 3-ethyl-1,2,4,5,7-pentamethoxy-6-methylnaphthalene (**2.48**) (10mg, 0.03mmol) in dry dichloromethane (1mL) was added boron tribromide (1M in dichloromethane, 0.15mL) at -78°C. The reaction mixture was warmed to room temperature and stirred for a further 48h, after which time water (2mL) was added prior to the addition of ethyl acetate (5mL). The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 5mL). The organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the deep red-purple solid. The spectroscopic data obtained for naphthazarin **1.16** was consistent with that reported previously.[12]

^{1}H NMR ($CDCl_3$) δ 1.14 (t, *J* = 8Hz, CH_2CH_3), 2.14 (s, 3H, CH_3), 2.67 (q, *J* = 8Hz, CH_2CH_3), 6.66 (s, 1H, O*H*), 6.68 (s, 1H, O*H*), 11.74 (s, 1H, O*H*), 13.47 (s, 1H, O*H*)

EIMS *m/z* 264 (100%, $M^{+\cdot}$), 249.0 (15%), 221.0 (52%), 190 (6%), 167 (5%), 137 (10%)

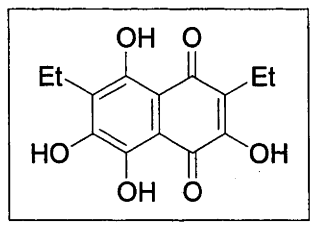
HREIMS *m/z* $M^{+\cdot}$ 264.06335 (calcd for $C_{13}H_{12}O_6$ 264.06334)

Melting Point 178-181°C (Lit. 180-184°C)[12]

UV/Vis (MeOH): 234, 264, 323, 510, 550 nm

Infrared ν_{max} (KBr): 3395 s, 2932 s, 1735 s, 1658 s, 1388 s, 1303 s, 1180 s, 1095 m, 810 w cm^{-1}

*The Synthesis of 2,7-diethyl-3,5,7,8-tetrahydroxy-1,4-naphthoquinone (**2.53**)*



To a stirred solution of naphthalene **2.50** (27.8mg, 0.10mmol) in dry dichloromethane (1mL) was added boron tribromide (1M in dichloromethane, 0.6mL) at -78°C. The reaction

mixture was warmed to room temperature and stirred for a further 48h, after which time water (2mL) was added prior to the addition of ethyl acetate (5mL). The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 5 mL). The organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the deep red-purple solid in quantitative yield. The spectroscopic data obtained for naphthazarin **2.53** was consistent with that reported previously.[13]

^{1}H NMR ($CDCl_3$) δ 1.03 (t, 6H, J = 7Hz, CH_3), 2.54 (q, 4H, J = 7Hz, CH_2)

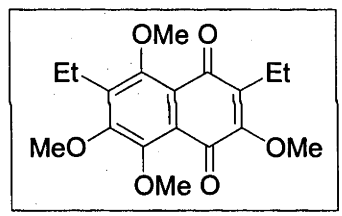
EIMS *m/z* 278 (100%, $M^{+\cdot}$), 263 (31%, $M^{+\cdot}$–Me), 250 (13%), 235 (39%), 207 (7%), 69 (18%)

HREIMS *m/z* $M^{+\cdot}$ 278.0787 (calcd for $C_{14}H_{14}O_6$ 278.0790)

Melting Point 183-184°C (Lit. 185-187°C)[13]

UV/Vis (MeOH): 233, 215 (sh), 323, 263 (sh) nm

The Synthesis of 3,6-diethyl-2,5,7,8-tetramethoxy-1,4-naphthoquinone (2.58)



To a solution of 3,6-diethyl-1,2,4,5,7,8-hexamethoxynaphthalene (**2.50**) (67mg, 0.2mmol) in freshly distilled, dry dioxane (2mL) was added freshly prepared silver(II) oxide (0.185g, 0.8mmol). The reaction mixture was then sonicated for 1 min to give a uniform dispersal of the oxidant. A freshly prepared solution of aqueous 6N nitric acid (0.2mL) was added and the reaction mixture stirred at room temperature for 2 min. A chloroform/water (8mL/2mL) mixture was then added and the mixture added to a separatory funnel. The organic layer was washed further with water (5mL) and then dried with magnesium sulfate, filtered and the solvent removed *in vacuo*. The product mixture was subjected to flash column chromatography (R_f 0.31, 15% ethyl acetate/ petroleum spirit) to give the desired naphthoquinone **2.58** as a yellow microcrystalline powder in 39% yield.

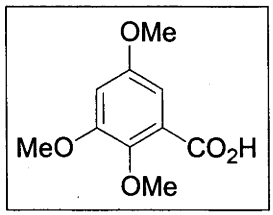
^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H, J = 6Hz, CH_2CH_3), 1.15 (t, 3H, J =6Hz, CH_2CH_3), 2.52 (q, 2H, J = 6Hz, CH_2CH_3), 2.70 (q, 2H, J = 6Hz, CH_2CH_3), 3.82 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3)

EIMS *m/z* 334 (96%, $M^{+\cdot}$), 320 (100%, $M^{+\cdot}$–CH_2), 305 (89%), 291 (56%), 277 (38%), 189 (66%), 149 (41%)

HREIMS *m/z* $M^{+\cdot}$ 334.1426 (calcd for $C_{18}H_{22}O_6$ 334.1416)

6.3 Experimental Procedures for Chapter Three

*The Synthesis of 2,3,5-trimethoxybenzoic acid (**3.13**)*



To a stirred solution of 2,3,5-trimethoxybromobenzene (**3.6**)[14] (0.60g, 2.43mmol) in dry THF (16mL) was added *n*-butyl lithium (1.34mL, 2.18M, 2.90mmol) at -78°C. The reaction mixture was stirred for 0.5h before the introduction of dry gaseous carbon dioxide. The gas was bubbled through the reaction mixture for 2h after which time it was warmed to room temperature prior to the addition of 5% hydrochloric acid (50mL). The solution was then extracted with ethyl acetate (3 x 50mL), the combined organic extracts were dried with magnesium sulfate, filtered and then concentrated *in vacuo* to give an oily residue. The residue was then purified by column chromatography (R_f 0.29, 50% ethyl acetate/ petroleum spirits) to give the white, crystalline benzoic acid in 92% yield. The melting point obtained for benzoic acid **3.13** was consistent with that reported previously.[15,16]

^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, OC*H*$_3$), 3.98 (s, 3H, OC*H*$_3$), 6.68 (d, 1H, *J* = 3Hz, aromatic C*H*), 7.12 (d, 1H, *J* = 3Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 55.8 (O*C*H$_3$), 56.1 (O*C*H$_3$), 62.3 (O*C*H$_3$), 104.6 (aromatic *C*H), 106.2 (aromatic *C*H), 121.8 (quaternary aromatic *C*), 142.5 (aromatic *C*-OMe), 152.8 (aromatic *C*-OMe), 156.4 (aromatic *C*-OMe), 165.4 (carbonyl *C*)

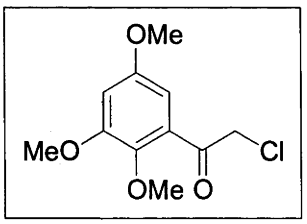
EIMS *m/z* 212 (62%, $M^{+\cdot}$), 197 (28%, $M^{+\cdot}$-Me), 169 (8%), 137 (13%), 118 (32%), 87 (100%)

HREIMS *m/z* $M^{+\cdot}$ 212.0687 (calcd for $C_{10}H_{12}O_5$ 212.0685)

Melting Point 94-5°C (Lit. 99.5-100.5°C)[15]

Infrared ν_{max} (KBr): 3448 m, 3109 m, 2954 m, 2846 w, 1735 s, 1604 m, 1434 s, 1365 s, 1257 m, 1188 s, 1049 s, 972 m cm^{-1}

*The Synthesis of 2-chloro-1-(2,3,5-trimethoxyphenyl)-ethanone (**3.19**)*



To a stirred solution of 2,3,5-trimethoxybenzoic acid (**3.13**) (0.34g, 1.60mmol) in toluene (6.2mL) was added thionyl chloride (0.30mL) at room temperature. The reaction mixture was heated under reflux for 1h then concentrated *in vacuo* to give the acid chloride **3.17**.

^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.69 (d, 1H, J = 3Hz, aromatic C*H*), 6.93 (d, 1H, J = 3Hz, aromatic C*H*)

CAUTION: Diazomethane is potentially explosive and the use of ground-glass joints or boiling chips should be avoided. This reaction was carried out in a purpose-built distillation apparatus.[5]

To a solution of potassium hydroxide (0.505g, 9mmol) in water (1mL) was added 96% ethanol (2.5mL) and the solution warmed to 60-65°C. A solution of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (2.165g, 10mmol) in diethyl ether (25mL) was then added dropwise, followed by the addition of diethyl ether (30mL). The ethereal diazomethane thus prepared was distilled into a solution of benzoyl chloride **3.17** in diethyl ether (5mL) at 0°C over a period of 0.5 h. The reaction mixture was warmed to room temperature and gently swirled for 5min. The solution was left for 1h and then the solvent was removed *in vacuo*.

The yellow residue was then added to a boiling suspension of silver(I) oxide (25mg, 0.11mmol) in methanol (2mL) and the reaction mixture heated under reflux for 0.5h. Another portion of silver(I) oxide (12.5mg, 0.05mmol) was then added to the reaction mixture and the heating continued for 15 min before the addition of another portion of silver(I) oxide (6mg, 0.03mmol). The reaction mixture was warmed to room temperature, the resulting suspension was filtered through celite and the filtrate was concentrated *in vacuo*. The crude product mixture was purified by flash column chromatography (R_f 0.43, 25% ethyl acetate/ petroleum spirits) to give 2-chloro-1-(2,3,5-trimethoxyphenyl)-ethanone (**3.19**) in 23% yield.

^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.89 (s, 3H, OC*H*$_3$), 4.81 (s, 2H, C*H*$_2$), 6.68 (d, 1H, *J* = 1Hz, aromatic C*H*), 6.82 (d, 1H, *J* = 1Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 50.4 (*C*H$_2$), 55.6 (O*C*H$_3$), 55.9 (O*C*H$_3$), 61.5 (O*C*H$_3$), 102.6 (aromatic *C*H), 105.6 (aromatic *C*H), 129.6 (aromatic *C*-C(O)CH_2Cl), 143.2 (aromatic *C*-OMe), 153.6 (aromatic *C*-OMe), 156.0 (aromatic *C*-OMe), 192.4 (carbonyl *C*)

gHSQC ^{1}H-^{13}C correlations: 3.81-55.6 ($^1J_{CH}$), 3.88-55.9 ($^1J_{CH}$), 3.89-61.5 ($^1J_{CH}$), 4.81-50.4 ($^1J_{CH}$), 6.68-105.6 ($^1J_{CH}$), 6.82-102.6 ($^1J_{CH}$)

gHMBC ^{1}H-^{13}C correlations: 4.81-192.4 ($^2J_{CH}$), 6.82-192.4 ($^3J_{CH}$)

EIMS *m/z* 246 (33%, $M^{+\cdot}$ $C_{11}H_{13}O_4{}^{37}Cl$), 244 (59%, $M^{+\cdot}$ $C_{11}H_{13}O_4{}^{35}Cl$), 240 (42%), 225 (34%), 195 (100%), 180 (27%), 152 (29%), 137 (26%)

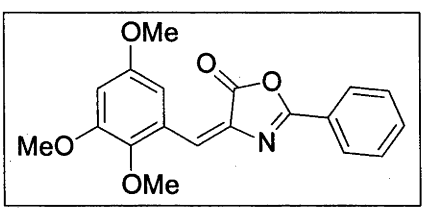
HREIMS *m/z* $M^{+\cdot}$ 246.0478 (calcd for $C_{11}H_{13}O_4{}^{37}Cl$ 246.0473)

m/z $M^{+\cdot}$ 244.0503 (calcd for $C_{11}H_{13}O_4{}^{35}Cl$ 244.0502)

Melting Point 101-102°C

Infrared ν_{max} (KBr): 3441 m, 2924 s, 2855 m, 1689 m, 1604 m, 1458 s, 1357 m, 1265 m, 1195 s, 1149 s cm^{-1}

*The Synthesis of 2-phenyl-4-(2,3,5-trimethoxybenzylidene)-4H-oxazol-5-one (**3.21**)*



A mixture of 2,3,5-trimethoxybenzaldehyde (**3.20**)[17] (0.35g, 1.8mmol), hippuric acid (0.32g, 1.78mmol), acetic anhydride (0.51mL) and anhydrous sodium acetate (0.15g, 1.83mmol) was heated at 100°C with stirring for 2h. The reaction mixture was then cooled, diluted with ethanol (2mL) and cooled to room temperature. The resulting suspension was then filtered and the residue washed with a further aliquot of cold ethanol (2mL) to give the bright yellow azlactone **3.21**. The azlactone **3.21** was recrystallised from ethyl acetate/petroleum spirits to give yellow prisms. The melting point obtained for azlactone **3.21** was consistent with that reported previously, the NMR data has not been reported previously.[18]

^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, OC*H*$_3$), 3.86 (s, 3H, OC*H*$_3$), 3.91 (s, 3H, OC*H*$_3$), 6.61 (d, 1H, *J* = 3Hz, aromatic C*H*), 7.50-7.60 (m, 3H, aromatic C*H*), 7.71 (s, 1H, vinylic *H*), 8.04-8.13 (m, 3H, aromatic C*H*)

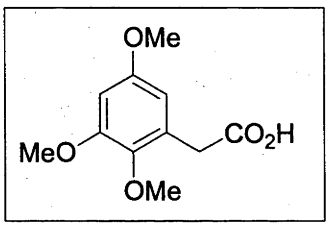
^{13}C NMR ($CDCl_3$) δ 55.6 (OCH_3), 55.9 (OCH_3), 62.2 (OCH_3), 104.3 (aromatic *C*H), 105.2 (aromatic *C*H), 125.6 (quaternary *C*), 125.9 (aromatic *C*H), 127.3 (quaternary *C*), 128.2 (2 x aromatic *C*H), 129.0 (2 x aromatic *C*H), 133.3 (aromatic *C*H), 133.5 (quaternary *C*), 144.7 (quaternary aromatic *C*), 153.3 (quaternary aromatic *C*), 155.9 (quaternary aromatic *C*), 163.3 (quaternary aromatic *C*), 167.6 (carbonyl *C*)

EIMS *m/z* 339 (69%, $M^{+\cdot}$), 155 (87%), 127 (68%), 105 (100%), 91 (64%), 77 (77%), 56 (78%)

HREIMS *m/z* $M^{+\cdot}$ 339.1107 (calcd for $C_{19}H_{17}NO_5$ 339.1107)

Melting Point 182-185°C (Lit. 181-183°C)[18]

*The Synthesis of 2,3,5-trimethoxyphenylacetic acid (**3.12**)*



2,3,5-Trimethoxyphenylacetic acid (**3.12**) was synthesized via a modified literature procedure.[18] A stirred solution of the 2-phenyl-4-(2,3,5-trimethoxybenzylidene)-4H-oxazol-5-one (**3.21**) (0.142g, 0.42mmol) in 10% sodium hydroxide (0.9mL) was heated under reflux for 1h. The reaction mixture was cooled to 0°C and diluted with ice-cold 40% sodium hydroxide (0.1mL). To this stirred solution was added a 15% aqueous solution of hydrogen peroxide (0.2mL) dropwise at 0°C. The solution was left at room temperature overnight. Concentrated hydrochloric acid (5mL) was then added to the solution prior to extraction with ethyl acetate (3 x 10mL). The combined organic extracts were then dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give a 1:1 mixture of 2,3,5-trimethoxy-phenylacetic acid (**3.12**) and benzoic acid as determined by ^{1}H NMR spectroscopy. The melting point obtained for phenyl acetic acid **3.12** was consistent with that reported previously, the NMR data has not been reported previously.

R_f 0.35 (25% ethyl acetate/ petroleum spirits)

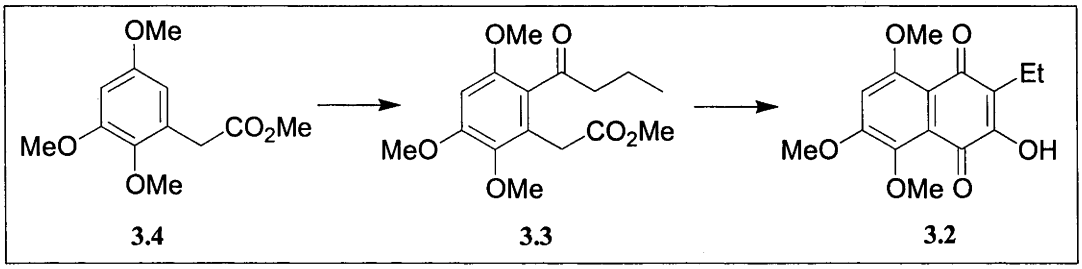
^{1}H NMR ($CDCl_3$) δ 3.64 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.30 (d, J = 2Hz, 1H, aromatic C*H*), 6.42 (d, J = 2Hz, 1H, aromatic C*H*)

EIMS *m/z* 226 (100%, $M^{+\cdot}$), 211 (89%, $M^{+\cdot}$-Me), 193 (15%), 167 (33%), 139 (43%), 123 (30%)

HREIMS *m/z* 226.0841 (calcd for 226.0841 $C_{11}H_{14}O_5$)

Melting Point 79-81 (Lit. 83°C)[18]

The Synthesis of 3-ethyl-2-hydroxy-5,7,8-trimethoxy-1,4-naphthoquinone (3.2)



2,3,5-Trimethoxyphenylacetic acid (**3.12**) (0.227g, 1.00mmol) was dissolved in methanol (0.9mL) containing sulfuric acid (0.02mL) and heated under reflux for 3h. The reaction mixture was then cooled to room temperature and the solvent concentrated *in vacuo*. Methyl 2,3,5-trimethoxyphenylacetate (**3.4**) was isolated in 89% yield as a colourless oil via flash column chromatography (R_f 0.49, 25% ethyl acetate/ petroleum spirits).

^{1}H NMR ($CDCl_3$) δ 3.64 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.31 (d, 1H, J = 2Hz, aromatic C*H*), 6.42 (d, 1H, J = 2Hz, aromatic C*H*)

To a solution of methyl 2,3,5-trimethoxyphenylacetate (**3.4**) (0.250g, 1.04mmol) in butyric anhydride (1mL) was added one drop of 40% aqueous perchloric acid at room temperature. The reaction mixture was stirred for 16h before the addition of aqueous 5% sodium hydrogen carbonate (50mL). The reaction mixture was extracted with ethyl acetate (3 x 20mL), the combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give the crude product mixture. The residue was then purified via flash column chromatography (R_f, 50% ethyl acetate/ petroleum spirits) to give the ketone **3.3** in 54% yield.

A solution of the ketone **3.3** (0.175g, 0.56mmol) in ethanol (3mL) was added dropwise to a solution of sodium ethoxide freshly prepared from sodium (0.03g, 1.3mmol) in ethanol (3mL). The reaction mixture was heated to reflux and stirred for 20min. Air was then bubbled through to the solution overnight at room temperature. The solvent was removed *in vacuo* and 1N sulfuric acid was then added (10mL). The reaction mixture

was extracted with ethyl acetate (3 x 10mL), the combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give the desired orange naphthoquinone. The quinone was then purified by column chromatography (25% ethyl acetate/ petroleum spirits) to give 3-ethyl-2-hydroxy-5,7,8-trimethoxy-1,4-naphthoquinone (**3.2**) in 47% yield.

^{1}H NMR ($CDCl_3$) δ 1.08 (t, 3H, *J* = 8Hz, CH_3), 2.54 (q, 2H, *J* = 8Hz, CH_2), 3.84 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.76 (s, 1H, aromatic *CH*), 7.28 (s, 1H, *OH*)

^{13}C NMR ($CDCl_3$) δ 12.7 (CH_2CH_3), 16.8 (CH_2CH_3), 56.2 (OCH_3), 56.8 (OCH_3), 61.1 (OCH_3), 103.1 (aromatic *C*H), 113.0 (quaternary aromatic *C*), 123.5 (quaternary aromatic *C*), 125.9 (quaternary aromatic *C*), 144.2 (quaternary aromatic *C*), 151.1 (quaternary aromatic *C*), 157.8 (quaternary aromatic *C*), 158.5 (quaternary aromatic *C*), 180.85 (carbonyl *C*), 180.89 (carbonyl *C*)

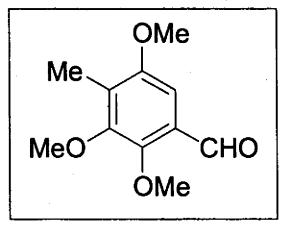
EIMS *m/z* 292 (88%, $M^{+\cdot}$), 277 (100%, $M^{+\cdot}$-Me), 265 (30%), 249 (66%), 223 (40%), 219 (21%)

HREIMS *m/z* $M^{+\cdot}$ 292.0950 (calcd for $C_{15}H_{16}O_6$ 292.0947)

Melting Point 47-49°C

Infrared ν_{max} (KBr): 3433 m, 2939 m, 2846 w, 1735 s, 1604 m, 1504 m, 1458 m, 1342 s, 1211 s, 1041 s cm^{-1}

The Synthesis of 2,3,5-trimethoxy-4-methylbenzaldehyde (3.29)



To a stirred solution of 2-hydroxy-3,5-dimethoxy-4-methyl-benzaldehyde (**3.33**) (2g, 10.19mmol) in dry *N*, *N*-dimethylformamide (20mL) was added dimethyl sulfate (0.72mL) and potassium carbonate (1.55g, 11.21mmol) at room temperature. The reaction mixture was stirred for 16h before it was added to an aqueous solution of 5% hydrochloric acid (50mL). Ethyl acetate (50mL) was added and the organic layer was separated using a separatory funnel. The aqueous layer was extracted with ethyl acetate (2 x 50mL) and the combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed on the high vacuum to give the desired benzaldehyde **3.29** in quantitative yield. The melting point and ^{1}H NMR data obtained for aldehyde **3.29** were consistent with the reported data.[19]

^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.98 (s, 1H, aromatic C*H*), 10.29 (s, 1H, C*H*O)

^{13}C NMR ($CDCl_3$) δ 9.6 (CH_3), 55.7 (OCH_3), 60.4 (OCH_3), 62.4 (OCH_3), 101.9 (aromatic *C*H), 126.9 (quaternary aromatic *C*), 129.6 (quaternary aromatic *C*), 151.2 (aromatic *C*-OMe), 152.0 (aromatic *C*-OMe), 154.4 (aromatic *C*-OMe), 189.3 (*C*HO)

EIMS *m/z* 210 (100%, $M^{+\cdot}$), 195 (85%, $M^{+\cdot}$–Me), 180 (17%), 167 (29%), 152 (23%), 139 (25%), 109 (28%)

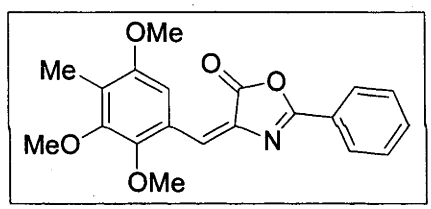
HREIMS *m/z* $M^{+\cdot}$ 210.0893 (calcd for $C_{11}H_{14}O_4$ 210.0892)

Melting Point 46-48 °C (Lit. 48-49°C)[19]

Infrared ν_{max} film (NaCl): 2939 m, 2847 w, 1681 m, 1589 m, 1466 s, 1411 m, 1280 m, 1126 s, 1026 w cm^{-1}

The Synthesis of

2-phenyl-4-(2,3,5-trimethoxy-4-methylbenzylidene)-4H-oxazol-5-one (3.37)



A stirred mixture of 2,3,5-trimethoxy-4-methylbenzaldehyde (**3.29**) (0.5g, 2.70mmol), hippuric acid (0.51g, 2.85mmol), acetic anhydride (0.82mL) and anhydrous sodium acetate (0.24g, 2.90mmol) was heated at 100°C for 2h. The reaction mixture was cooled to room temperature, diluted with ethanol (4mL) and filtered to give a orange/yellow powder. (0.190g, 0.53mmol). The solid was purified by recrystallisation from ethyl acetate/ petroleum spirits to give yellow prisms.

^{1}H NMR ($CDCl_3$) δ 2.19 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 7.46-7.62 (m, 3H, aromatic C*H*), 7.69 (s, 1H, aromatic C*H*), 8.06-8.12 (m, 2H, aromatic C*H*), 8.24 (s, 1H, vinylic C*H*)

^{13}C NMR ($CDCl_3$) δ 9.5 (CH_3), 55.6 (OCH_3), 60.4 (OCH_3), 61.9 (OCH_3), 107.6 (aromatic *C*H), 124.7 (quaternary *C*), 125.7 (quaternary *C*), 126.5 (aromatic *C*H), 127.6 (quaternary *C*), 128.0 (2 x aromatic *C*H), 128.9 (2 x aromatic *C*H), 132.4 (quaternary aromatic *C*), 133.1 (vinyl *C*H), 148.6 (quaternary aromatic *C*), 151.7 (quaternary aromatic *C*), 154.3 (quaternary aromatic *C*), 162.7 (quaternary *C*), 167.7 (carbonyl *C*)

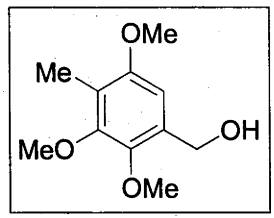
EIMS *m/z* 353 (58%, $M^{+\cdot}$), 205 (2%), 167 (3%), 105 (100%), 77 (32%)

HREIMS *m/z* $M^{+\cdot}$ 353.1266 (calcd for $C_{20}H_{19}NO_5$ 353.1263)

Melting Point 153-154°C

Infrared ν_{max} (KBr): 3441 s, 2939 w, 1790 m, 1651 s, 1589 s, 1458 w, 1411 w, 1319 w, 1280 s, 1226 w, 1164 m, 1110 s 1018 w cm^{-1}

*The Synthesis of 2,3,5-trimethoxy-4-methylbenzyl alcohol (**3.38**)*



To a solution of 2,3,5-trimethoxy-4-methylbenzaldehyde (**3.29**) (1.17g, 5.90mmol) in dry THF (15mL) and methanol (4mL) was added sodium borohydride (0.06g, 1.59mmol) at 0°C. The solution was stirred for 1.5h at 0°C, following which a solution of 5% aqueous hydrochloric acid (50mL) was added prior to the addition of ethyl acetate (20mL). The organic layer was separated using a separatory funnel and the aqueous layer was re-extracted with ethyl acetate (2 x 20mL). The combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to give the pale oil in 99% yield.

^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.64 (s, 2H, CH_2), 6.58 (s, 1H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 8.7 (CH_3), 55.5 (OCH_3), 60.1 (OCH_3), 60.7 (CH_2), 60.8 (OCH_3), 105.1 (aromatic *C*H), 120.0 (aromatic *C*-Me), 131.2 (aromatic *C*-CH_2OH), 144.4 (aromatic *C*-OMe), 151.4 (aromatic *C*-OMe), 154.0 (aromatic *C*-OMe)

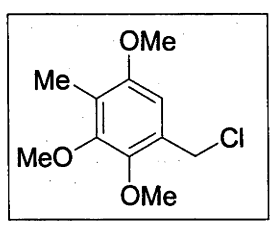
EIMS *m/z* 212 (100%, $M^{+\cdot}$), 197 (48%, $M^{+\cdot}$-Me), 182 (23%), 154 (40%), 137 (89%), 122 (15%), 109 (30%)

HREIMS *m/z* $M^{+\cdot}$ 212.1047 (calcd for $C_{11}H_{16}O_4$ 212.1049)

Anal. Calc. For $C_{11}H_{16}O_4$: C, 62.25, H, 7.60. Found: C, 62.41, H, 7.54%.

Infrared ν_{max} (KBr): 3425 s, 2939 s, 1596 w, 1465 s, 1404 s, 1326 w, 1234 m, 1126 s, 1033 m cm^{-1}

*The Synthesis of 2,3,5-trimethoxy-4-methylbenzyl chloride (**3.39**)*



A solution of 2,3,5-trimethoxy-4-methylbenzyl alcohol (**3.38**) (1.24g, 5.84mmol) in thionyl chloride (1.36g, 11.43mmol) was

stirred at room temperature for 1h. The excess thionyl chloride was removed *in vacuo* prior to the addition of ethyl acetate (20mL) and water (20mL). The organic layer was separated using a separatory funnel and washed with brine (20mL). The organic layer was then dried with magnesium sulfate, filtered and the filtrate was concentrated *in vacuo*. The product was purified by flash column chromatography (R_f 0.42, 15% ethyl acetate/ petroleum spirits) to give 2,3,5-trimethoxy-4-methylbenzyl chloride (**3.39**) as a pale yellow oil in 96% yield.

^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H, C*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 4.62 (s, 2H, C*H*$_2$), 6.59 (s, 1H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 9.0 (*C*H$_3$), 41.5 (*C*H$_2$), 55.6 (OCH$_3$), 60.2 (OCH$_3$), 61.2 (OCH$_3$), 106.6 (aromatic *C*H), 122.0 (quaternary aromatic *C*), 128.1 (quaternary aromatic *C*), 145.3 (quaternary aromatic *C*), 151.9 (quaternary aromatic *C*), 154.1 (quaternary aromatic *C*)

EIMS *m/z* 232 (80%, $C_{11}H_5{}^{37}ClO_3$), 230 (100%, $M^{+\cdot}$ $C_{11}H_5{}^{35}ClO_3$), 215 (72%, $M^{+\cdot}$-Me), 195 (76%, $M^{+\cdot}$-^{35}Cl), 179 (75%), 165 (39%), 152 (30%)

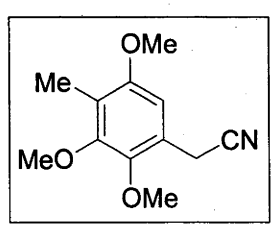
HREIMS *m/z* $M^{+\cdot}$ 232.0681 (calcd for $C_{11}H_{15}O_3{}^{37}Cl$ 230.0680)

m/z $M^{+\cdot}$ 230.0713 (calcd for $C_{11}H_{15}O_3{}^{35}Cl$ 230.0710)

Anal. Calc. for $C_{11}H_{15}ClO_3$: C, 57.27, H, 6.55. Found: C, 57.26, H, 6.48

Infrared ν_{max} (KBr): 2939 s, 2839 m, 1597 w, 1466 s, 1404 s, 1334 m, 1265 s, 1188 w, 1134 s, 1087 s, 1033 s, 941 w cm^{-1}

*The Synthesis of 2,3,5-trimethoxy-4-methylbenzyl cyanide (**3.40**)*



To a solution of 2,3,5-trimethoxy-4-methylbenzyl chloride (**3.39**) (1.30g, 5.64mmol) in dimethyl sulfoxide (23mL) was added potassium cyanide (0.50g, 7.69mmol) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured onto ice and the resultant solution was extracted with ethyl acetate (3 x 20mL). The combined organic extracts were dried with magnesium sulfate, filtered and the solvent concentrated under reduced pressure to give 2,3,5-trimethoxy-4-methylbenzyl cyanide (**3.40**) as a white solid in 99% yield.

^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H, C*H*$_3$), 3.68 (s, 2H, C*H*$_2$CN), 3.78 (s, 3H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.83 (s, 3H, OC*H*$_3$), 6.55 (s, 1H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 8.9 (CH_3), 18.6 (CH_2CN), 55.9 (OCH_3), 60.2 (OCH_3), 60.7 (OCH_3), 105.7 (aromatic *C*H), 118.4 (CH_2CN), 120.7 (quaternary aromatic *C*), 121.4 (quaternary aromatic *C*), 144.7 (aromatic *C*-OMe), 152.0 (aromatic *C*-OMe), 154.3 (aromatic *C*-OMe)

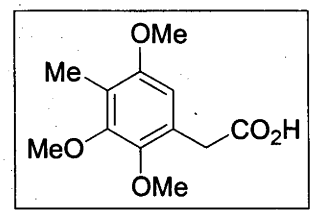
EIMS *m/z* 221 (55%, $M^{+\cdot}$), 206 (28%, $M^{+\cdot}$-Me), 196 (27%), 178 (10%), 165 (6%)

HREIMS *m/z* $M^{+\cdot}$ 221.10561 (calcd for $C_{12}H_{15}NO_3$ 221.10519)

Melting Point 59-60°C

Infrared ν_{max} (KBr): 3441 s, 2939 s, 2250 m, 1604 w, 1465 m, 1404 m, 1242 w, 1134 s, 1080 m, 1033 m cm^{-1}

*The Synthesis of 2,3,5-trimethoxy-4-methylphenylacetic acid (**3.41**)*



Method A

To a solution of 2,3,5-trimethoxy-4-methylbenzyl cyanide (**3.40**) (1.27g, 5.74mmol) in glacial acetic acid (25mL) was added water (8mL) and concentrated sulfuric acid (2.5mL). The reaction mixture was heated to reflux for 22h and then cooled to room temperature. The mixture was poured onto ice and the resultant solution was extracted with ethyl acetate (3 x 25mL). The combined organic extracts were washed with brine (30mL), dried with magnesium sulfate, filtered and the solvent concentrated under reduced pressure.

Method B

A solution of 2-phenyl-4-(2,3,5-trimethoxy-4-methylbenzylidene)-4H-oxazol-5-one (**3.37**) (1.26g, 3.57mmol) in 10% sodium hydroxide (7.7mL) was heated under reflux for 5h. The reaction mixture was cooled to 0°C and diluted with ice-cold 10% sodium hydroxide (2mL). To this was added, with stirring, a 15% hydrogen peroxide solution (1mL) and the temperature was kept below 15°C. The reaction mixture was stirred at room temperature overnight, then acidified with concentrated hydrochloric acid. The resultant solution was then extracted with ethyl acetate (3 x 10mL). The combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed *in vacuo* prior to purification via flash column chromatography (R_f 0.46, 25% ethyl

acetate/ petroleum spirits) to give the phenylacetic acid **3.41** as a colourless oil in 86% yield.

^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H, CH_3), 3.64 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.45 (s, 1H, aromatic C*H*)

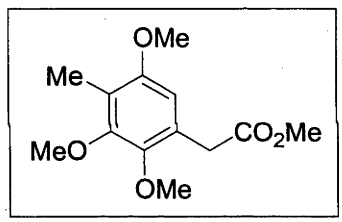
^{13}C NMR ($CDCl_3$) δ 8.6 (*C*H_3), 35.8 (*C*H_2), 55.8 (O*C*H_3), 60.3 (O*C*H_3), 60.7 (O*C*H_3), 107.3 (aromatic *C*H), 120.6 (quaternary aromatic *C*), 124.3 (quaternary aromatic *C*), 145.3 (aromatic *C*-OMe), 151.8 (aromatic *C*-OMe), 154.0 (aromatic *C*-OMe), 177.8 (carbonyl *C*)

EIMS *m/z* 240 (100%, $M^{+\cdot}$), 225 (90%, $M^{+\cdot}$-Me), 207 (24%), 181 (68%), 137 (38%)

HREIMS *m/z* $M^{+\cdot}$ 240.1000 (calcd for $C_{12}H_{16}O_5$ 240.0998)

Infrared ν_{max} (KBr): 3441 s, 2932 s, 1712 m, 1635 w, 1465 m, 1404 m, 1242 m, 1126 s, 1087 m, 1033 m cm^{-1}

*The Synthesis of methyl 2,3,5-trimethoxy-4-methylphenylacetate (**3.28**)*



A stirred solution of 2,3,5-trimethoxy-4-methyl-phenylacetic acid (**3.41**) (1.13g, 4.69mmol) in methanol (10mL) containing 2% sulfuric acid (0.2mL) was heated under reflux for 5h. The reaction mixture was then cooled and concentrated under reduced pressure to give methyl 2,3,5-trimethoxy-4-methylphenylacetate (**3.28**) as the crude product. Purification of the ester was carried out by flash column chromatography (R_f 0.45, 15% ethyl acetate/ petroleum spirits) and isolated as a colourless oil in 77% yield.

^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 3.67 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.45 (s, 1H, aromatic C*H*)

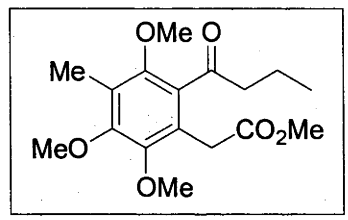
^{13}C NMR ($CDCl_3$) δ 8.8 (*C*H_3), 35.5 (*C*H_2), 52.0 (O*C*H_3), 55.7 (O*C*H_3), 60.2 (O*C*H_3), 60.6 (O*C*H_3), 107.1 (aromatic *C*H), 120.1 (quaternary aromatic *C*), 124.8 (quaternary aromatic *C*), 145.2 (aromatic *C*-OMe), 151.7 (aromatic *C*-OMe), 153.9 (aromatic *C*-OMe), 172.3 (*C*O_2CH_3)

EIMS *m/z* 254 (100%, $M^{+\cdot}$), 239 (82%, $M^{+\cdot}$-Me), 221 (21%), 195 (25%), 180 (34%), 179 (29%), 69 (67%)

HREIMS *m/z* $M^{+\cdot}$ 254.1154 (calcd for $C_{13}H_{18}O_5$ 254.1154)

Infrared ν_{max} film (NaCl): 2939 s, 1736 s, 1596 w, 1466 s, 1404 s, 1319 m, 1242 s, 1126 s, 1087 m, 1033 m cm^{-1}

*The Synthesis of Methyl 2-butyryl-3,5,6-trimethoxy-4-methylphenylacetate (**3.1**)*



To a solution of methyl 2,3,5-trimethoxy-4-methylphenyl acetate (**3.28**) (0.92g, 3.60mmol) in butyric anhydride (3.5mL) was added 40% aqueous perchloric acid (0.02mL) at room temperature. The reaction mixture was stirred for 16h prior to the slow addition of 5% sodium hydrogen carbonate (10mL). The solution was extracted with ethyl acetate (3 x 20mL), the combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give the crude product. The residue was then purified using column chromatography (R_f 0.34, 50% ethyl acetate/ petroleum spirits) to give the ketone **3.1** as a colourless oil in 35% yield.

^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, *J* = 7Hz, $CH_2CH_2CH_3$), 1.66 (s, 2H, *J* = 7Hz, $CH_2CH_2CH_3$), 2.16 (aromatic C-CH_3), 2.80 (t, 2H, *J* = 7Hz, $CH_2CH_2CH_3$), 3.61 (s, 3H, OCH_3), 3.66 (s, 2H, CH_2CO_2Me), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3)

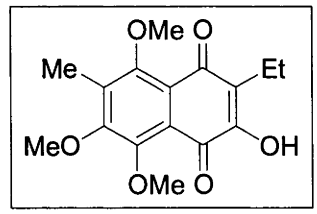
^{13}C NMR ($CDCl_3$) δ 13.7 ($CH_2CH_2CH_3$), 17.1 ($CH_2CH_2CH_3$), 31.6 (CH_2CO_2Me), 46.6 ($CH_2CH_2CH_3$), 52.0 (aromatic C-CH_3), 60.2 (OCH_3), 60.3 (OCH_3), 60.4 (OCH_3), 62.2 (OCH_3), 123.0 (quaternary aromatic *C*), 125.3 (quaternary aromatic *C*), 132.2 (quaternary aromatic *C*), 148.2 (aromatic *C*-OMe), 151.6 (aromatic *C*-OMe), 152.3 (aromatic *C*-OMe), 171.8 (CO_2Me), 207.4 (*C*(O)Pr)

EIMS *m/z* 324 (84%, $M^{+\cdot}$), 288 (42%), 281 (67%), 265 (35%), 254 (92%), 253 (100%), 239 (43%)

HREIMS *m/z* $M^{+\cdot}$ 324.1575 (calcd for $C_{17}H_{24}O_6$ 324.1573)

Infrared ν_{max} (KBr): 2939 s, 1735 s, 1589 w, 1461 m, 1404 m, 1327 w, 1242 m, 1169 s, 1091 s, 1018 m cm^{-1}

The Synthesis of 2-ethyl-3-hydroxy-5,6,8-trimethoxy-7-methyl-1,4-naphthoquinone (1.63)



A solution of ketone **3.1** (0.40g, 1.25mmol) in dry ethanol (7mL) was added dropwise to a solution of sodium ethoxide freshly prepared from sodium (0.067g, 2.91mmol) in ethanol (7mL). The reaction mixture was heated to reflux and stirred for 20min. Air was then bubbled through to the solution overnight at room temperature, following which the solvent was removed *in vacuo*. To the residue was added 1N sulfuric acid (5mL). The organic layer was then separated and the aqueous layer was re-extracted with ethyl acetate (3 x 10mL). The combined organic extracts were dried with magnesium sulfate, filtered and the solvent removed under reduced pressure to give the orange naphthoquinone. The quinone was then purified by flash column chromatography (R_f 0.35, 25% ethyl acetate/ petroleum spirits) to give the product in 56% yield. The spectroscopic data obtained for naphthoquinone **1.63** was identical to that reported previously.[20,21]

^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, *J* = 8Hz, CH_2CH_3), 2.29 (s, 3H, CH_3), 2.57 (t, 2H, *J* = 8Hz, CH_2CH_3), 3.83 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.42 (s, 1H, O*H*)

^{13}C NMR ($CDCl_3$, 500MHz) δ 10.1 (CH_3), 12.7 (CH_2CH_3), 16.8 (CH_2CH_3), 60.9 (OCH_3), 61.15 (OCH_3), 61.22 (OCH_3), 121.1 (quaternary *C*), 125.2 (quaternary *C*), 136.9 (quaternary *C*), 150.7 (quaternary *C*), 151.6 (quaternary *C*), 156.2 (quaternary *C*), 157.1 (quaternary *C*), 180.4 (carbonyl *C*), 183.7 (carbonyl *C*)

(N.B. One quaternary carbon signal was not observed.)

EIMS *m/z* 306 (100%, $M^{+\cdot}$), 292 (59%, $M^{+\cdot}$-CH_2), 277 (71%, $M^{+\cdot}$-Et), 263 (26%), 233 (15%), 168 (100%), 153 (52%)

HREIMS *m/z* $M^{+\cdot}$ 306.1101 (calcd for $C_{16}H_{18}O_6$ 306.1103)

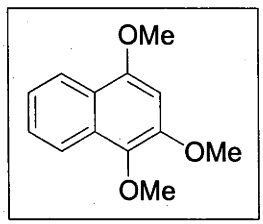
Melting Point 105-106 (Lit. 106-108°C)[21]

Anal. Calc. for $C_{16}H_{18}O_6$: C, 62.74, H, 5.92. Found: C, 62.57, H, 6.23%.

Infrared ν_{max} (KBr): 3356 s, 2932 s, 1651 m, 1466 s, 1404 s, 1350 s, 1288 s, 1242 m, 1072 s cm^{-1}

6.4 Experimental Procedures for Chapter Four

The synthesis of 1,2,4-trimethoxynaphthalene (4.3)



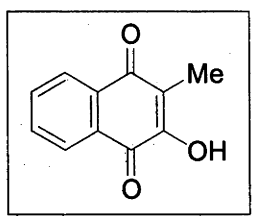
To a stirred solution of lawsone (**1.3**) (1.75g, 10mmol) in THF (25mL) and water (10mL) was added tetrabutylammonium bromide (0.375g, 1.16mmol) at room temperature. A solution of sodium dithionite (10.45g) in water (50mL) was added to this solution and the reaction mixture stirred for 10 min, after which time an aqueous solution of sodium hydroxide was added (1M, 230mL) and the reaction mixture stirred for 5 min. Dimethyl sulfate (20mL) was added to the reaction mixture and the solution stirred for 18h at room temperature. Ethyl acetate (100mL) was added to the solution and the organic layer was separated using a separatory funnel. The aqueous layer was extracted with ethyl acetate (2 x 100mL) and the organic layers were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo*. The product was purified by flash column chromatography (R_f 0.53, 15% ethyl acetate/ petroleum spirits) to give 1,2,4-trimethoxynaphthalene (**4.3**) in 73% yield. The spectroscopic data obtained for naphthalene **4.3** was consistent with that previously reported, the ^{13}C NMR data has not been reported previously.[22]

^{1}H NMR ($CDCl_3$) δ 3.96 (s, 3H, OC*H*$_3$), 4.01 (s, 3H, OC*H*$_3$), 4.03 (s, 3H, OC*H*$_3$), 6.68 (s, 1H, aromatic C*H*), 7.35-7.56 (m, 2H, aromatic C*H*), 8.05-8.22 (m, 2H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 55.7 (O*C*H$_3$), 57.3 (O*C*H$_3$), 61.2 (O*C*H$_3$), 95.2 (aromatic *C*H), 120.9 (aromatic *C*H), 121.3 (quaternary aromatic *C*), 122.0 (aromatic *C*H), 123.3 (aromatic *C*H), 126.8 (aromatic *C*H), 129.2 (quaternary aromatic *C*), 136.6 (aromatic *C*-OMe), 148.1 (aromatic *C*-OMe), 152.4 (aromatic *C*-OMe)

EIMS *m/z* 218 (85%, $M^{+\cdot}$), 203 (100%, $M^{+\cdot}$-Me), 175 (71%, $M^{+\cdot}$-Me-OH)

The synthesis of phthiocol (1.7)



To a solution of 1,2,4-trimethoxynaphthalene (**4.3**) (0.89g, 4.1mmol) in THF (100mL) was added a solution of *n*-butyl lithium (1.5 M in hexanes, 7.3 eq.) and

tetramethylethylenediamine (6mL) at –78 °C and the solution stirred for 0.5h, after which time methyl iodide (12mL, excess) was added and the solution stirred for 0.5h. The reaction mixture was warmed to room temperature and poured onto 5% HCl (100mL). Ethyl acetate (100mL) was added and the organic layer was separated using a separatory funnel. The aqueous layer was extracted with ethyl acetate (2 x 100mL) and the combined organic fractions were then dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to reveal the crude product mixture. The desired product 1,2,4-trimethoxy-3-methyl-naphthalene (**4.4**) was isolated in 77% yield by flash column chromatography (R_f 0.51,15% ethyl acetate/ petroleum spirits)

^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, C*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.97 (s, 3H, OC*H*$_3$), 3.99 (s, 3H, OC*H*$_3$), 7.40- 7.46 (m, 2H, aromatic C*H*), 8.01-8.13 (m, 2H, aromatic C*H*)

To a solution of 1,2,4-trimethoxy-3-methylnaphthalene (**4.4**) (0.63g, 2.7mmol) in dichloromethane at –78 °C was added a solution of boron tribromide (1M in dichloromethane, 14.12mL) and the reaction mixture was gradually warmed to room temperature. The solution was stirred for 96h at room temperature, after which time the reaction was quenched with water. The organic layer was isolated, dried with magnesium sulfate, filtered and the solvent removed *in vacuo* to reveal the crude product which was purified by flash column chromatography (R_f 0.31, 15% ethyl acetate/ petroleum spirits) and isolated in 96% yield. The melting point obtained for phthiocol (**1.7**) was consistent with that reported previously.[23]

^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H, C*H*$_3$), 7.31 (bs, 1H, O*H*), 7.72-7.82 (m, 2H, aromatic C*H*), 7.93-7.99 (m, 2H, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 8.8 (*C*H$_3$), 120.6 quinone *C*-Me), 126.2 (aromatic *C*H), 126.8 (aromatic *C*H), 129.4 (quaternary *C*), 132.9 (aromatic *C*H), 133.0 (quaternary *C*), 134.9 (aromatic *C*H), 153.2 (quinone *C*-OH), 181.2 (carbonyl *C*), 185.2 (carbonyl *C*)

EIMS *m/z* 188 (100%, $M^{+\cdot}$), 174 (27%, $M^{+\cdot}$-Me), 160 (40%, $M^{+\cdot}$-CO)

HREIMS *m/z* $M^{+\cdot}$ 188.0469 (calcd for $C_{11}H_8O_3$ 188.0473)

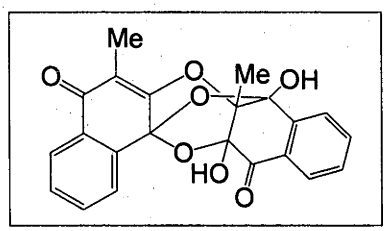
Melting Point 171-173°C (Lit. 172-173°C)[23]

Infrared ν_{max} (KBr): 3341 s, 1728 s, 1658 s, 1589 m, 1458 m, 1396 m, 1342 m, 1280 s, 1211 s, 1072 s cm^{-1}

The Synthesis of Phthiocol Dimers

To a stirred solution of phthiocol (**1.7**) in boiling glacial acetic acid (35mL) was added lead(IV) oxide (2.53g, 10.6mmol). The reaction mixture was stirred at 120°C for 10 min prior to filtering through a plug of celite. The volume of the filtrate was then reduced *in vacuo* and the resultant residue was separated chromatographically (50% ethyl acetate/ petroleum spirits) to give compound **4.17** and 2-methyl-3-(2-methyl-1,3-dioxo-indan-2-yloxy)-1,4-naphthoquinone **4.18** in 7% and 42% yield respectively.

Compound 4.17



R_f (0.64, 50% ethyl acetate/ petroleum spirits)

^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 5.96 (s, 1H, O*H*), 6.70 (s, 1H, O*H*), 7.18-7.31 (m, 3H, aromatic C*H*), 7.49 (t, 1H, *J* = 8Hz, aromatic C*H*), 7.65 (t, 1H, *J* = 8Hz, aromatic C*H*), 7.88-7.95 (m, 2H, aromatic C*H*), 8.04 (d, 1H, *J* = 8Hz, aromatic C*H*)

^{13}C NMR ($CDCl_3$) δ 6.5 (CH_3), 14.5 (CH_3), 79.9 (quaternary *C*), 88.5 (quaternary *C*), 94.2 (quaternary *C*), 95.8 (quaternary *C*), 109.0 (quaternary *C*), 125.1 (quaternary *C*), 125.4 (aromatic *C*H), 125.8 (aromatic *C*H), 126.1 (aromatic *C*H), 128.3 (aromatic *C*H), 129.6 (quaternary *C*), 129.9 (aromatic *C*H), 130.0 (aromatic *C*H), 132.0 (aromatic *C*H), 133.8 (quaternary *C*), 135.8 (aromatic *C*H), 141.0 (quaternary *C*), 160.7 (quaternary *C*), 183.5 (carbonyl *C*), 187.5 (carbonyl *C*)

EIMS *m/z* 381 (14%), 380 (21%), 346 (29%), 273 (12%), 245 (24%), 205 (33%), 188 (71%), 160 (43%)131 (38%), 91 (100%)

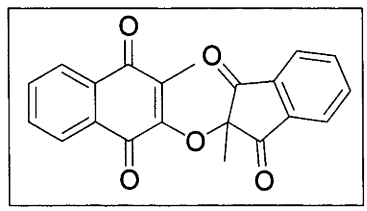
HREIMS *m/z* $M^{+\cdot}$ 381.2283 (calcd for $C_{21}H_{33}O_6$ 381.2277)

m/z $M^{+\cdot}$ 346.0838 (calcd for $C_{21}H_{14}O_5$ 346.0841)

Melting Point 180°C

Infrared ν_{max} (KBr): 3310 s, 1705 s, 1670 s, 1647 s, 1600 s, 1577 w, 1382 w, 1346 m, 1276 m, 1226 s, 1190 w, 1028 m cm^{-1}

2-Methyl-3-(2-methyl-1,3-dioxo-indan-2-yloxy)-1,4-naphthoquinone ***4.18***



R_f (0.48, 50% ethyl acetate/ petroleum spirits)

^{1}H NMR ($CDCl_3$) δ 1.74 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 7.5-8.1 (m, 8H, aromatic *H*)

^{13}C NMR ($CDCl_3$) δ 9.8 (CH_3), 23.4 (CH_3), 84.1 (quaternary *C*), 124.1 (2 x aromatic *C*H), 126.3 (2 x aromatic *C*H), 130.3 (quaternary *C*), 130.5 (quaternary *C*), 132.0 (quaternary *C*), 133.0 (aromatic *C*H), 134.2 (aromatic *C*H), 135.9 (2 x aromatic *C*H), 138.6 (quaternary *C*), 152.8 (quaternary *C*), 181.1 (carbonyl *C*), 185.0 (carbonyl *C*), 195.6 (2 x carbonyl *C*)

(N.B. One quaternary C is not accounted for.)

gHMBC (^{1}H-^{13}C correlations): 1.74-195.6 ($^3J_{CH}$), 1.74-84.1 ($^2J_{CH}$), 2.27-185.0 ($^3J_{CH}$), 2.27-152.8 ($^3J_{CH}$)

gHMQC (^{1}H-^{13}C correlations): 9.8-2.27 ($^1J_{CH}$), 23.4-1.74 ($^1J_{CH}$)

EIMS *m/z* 347 (100%, M^+H), 309 (11%), 305 (28%), 287 (70%), 225 (10%), 213 (11%), 177 (12%)

HREIMS *m/z* M^+ 346.0839 (calcd for $C_{21}H_{14}O_5$ 346.0841)

Melting Point 122.5°C

Infrared ν_{max} (KBr): 2928 m, 1759 m, 1724 s, 1659 s, 1602 w, 1332 m, 1263 m, 1205 s cm^{-1}

The following are general procedures utilised in the work described in Chapter Four:

General Procedure for Photolysis

A solution of the quinone (2.9 mmol) in distilled water (750 mL) was irradiated using a 100W tungsten filament lamp at 80°C. Aliquots of the solutions were removed at various time intervals so as to monitor the reaction. Ethyl acetate (2mL) was added to the aqueous aliquot (2mL) and the mixture was shaken vigorously. The organic layer was separated using a separatory funnel, dried using magnesium sulfate, filtered and the solvent removed *in vacuo*. The resultant residue was then analysed by HPLC.

The sunlight experiments involved the exposure of the quinone (0.1 mmol) in methanol (2 mL) to sunlight on the roof of the Department of Chemistry, The Australian National

University. Aliquots were removed at various time intervals, the solvent removed under reduced pressure and the resultant residue was analysed by HPLC.

General Procedure for Electrolysis

To a solution of quinone (0.1 mmol) in degassed acetonitrile (25 mL) was added ferrocene (1.75 mg) and tetrabutylammonium hexaflurophosphate (855 mg). The solution was then placed in an electrochemical cell. A three-electrode potentiostat consisting of a Princeton Applied Research Electrochemistry System (PAR 170), a Tacussel platinum disc electrode as the working electrode, a platinum wire quasi-reference electrode and a platinum wire as the auxiliary electrode was employed and a 2.2V current was applied for 16h. An aliquot of this solution was removed, the volume reduced *in vacuo* and the resultant reside was analysed by HPLC.

General Procedure for Potassium Persulfate

Method A

To a solution of quinone (0.28mmol) in distilled water (10mL) and 4 N NaOH (1.4mL) at 95°C was added potassium persulfate (5.5mmol) in distilled water (5mL) over 0.5 h. The reaction mixture was stirred for a further 90 min at 95°C then allowed to cool to room temperature prior to the addition of ethyl acetate (20mL). The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 20mL). The organic fractions were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo*. The resultant residue was then analysed by HPLC.

Method B

To a stirred solution of the quinone (2.0mmol) in distilled water (200mL) at 100°C was added persulfate (3.0mmol) in distilled water (100mL) over 15 min. The reaction mixture was stirred at 100 °C for 60 min then allowed to cool to room temperature prior to the addition of ethyl acetate (20mL). The organic layer was then separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 20mL).

The organic fractions were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo*. The resultant residue was then subjected to HPLC analysis.

General Procedure for Ammonium Metavanadate

Ammonium vanadate (76mg, 0.65mmol) was dissolved in boiling water (2.6mL) and cooled to room temperature. To this, a solution of 40% aqueous perchloric acid (0.72mL) in water (0.77mL) was added gradually, with stirring. The resultant solution was added to a solution of the quinone (0.46mmol) in acetone (2mL) at room temperature. The reaction mixture was stirred at room temperature for 16h prior to the addition of ethyl acetate (2mL). The organic layer was separated using a separatory funnel and the aqueous layer was extracted with ethyl acetate (2 x 2mL). The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The resultant residue was then analysed by HPLC.

General Procedure for Lead(IV) Oxide

To a stirred solution of the quinone (0.53mmol) in boiling glacial acetic acid (3mL) was added lead(IV) oxide (0.25g, 1.1mmol). The reaction mixture was stirred at 120°C for a given period of time prior to filtering through celite. The volume of the filtrate was then reduced *in vacuo* and the resultant residue was analysed by HPLC.

General Procedure for Ceric Ammonium Nitrate (CAN)

To a stirred solution of the quinone (2.8mmol) in acetonitrile : water (3:1) (5mL) at 0°C was added CAN (0.29g, 0.53mmol). The reaction was allowed to stir for 1h at 0°C after which time $CHCl_3$ (3mL) was added. The organic layer was separated using a separatory funnel, dried with magnesium sulfate, filtered and the solvent removed under reduced pressure prior to HPLC analysis.

General Procedure for Potassium Ferricyanide

To a solution of the quinone (0.04mmol) in distilled water (2mL) was added potassium ferricyanide (23mg, 0.07mmol). A solution of potassium carbonate (0.143g, 1.03mmol) in distilled water (2mL) was added and the reaction mixture stirred at room temperature.

Aliquots were removed so as to monitor the reaction. Ethyl acetate (2mL) was added to the aqueous aliquot (2mL) and the mixture was vigorously shaken. The organic layer was separated using a separatory funnel, dried using magnesium sulfate, filtered and the solvent removed *in vacuo*. The resultant residue was then analysed by HPLC.

General Procedure for Manganese Oxide

To a stirred solution of the quinone (0.04mmol) in dichloromethane (10mL) was added activated manganese dioxide (52mg, 0.6mmol) at room temperature. The solution was monitored by filtering an aliquot through a plug of silica and removing the solvent *in vacuo*. The resultant residue was then analysed by HPLC.

General Procedure for Ferric Chloride

To a solution of quinone (0.58mmol) in distilled water (4mL) was added ferric chloride hexahydrate (0.40g, 1.48mmol) and the solution stirred at to 65°C. The reaction mixture was monitored over time through the removal of an aliquot (0.5mL). Ethyl acetate (1mL) was added to the aqueous solution and the mixture was shaken vigorously. The organic layer was separated using a separatory funnel, dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The resultant residue was then analysed by HPLC.

General Procedure for Silver Oxide/ Nitric Acid

To a solution of the quinone (0.6mmol) in chloroform (10mL) was added triethylamine (0.02mL) and silver(II) oxide (0.15g, 1.2mmol) and the reaction mixture was stirred at room temperature for 24h, after which time the solution was filtered, the solvent removed *in vacuo* and 65% nitric acid (4mL) added to the crude product mixture. The solution was stirred at room temperature for 3 min, diluted with distilled water (10mL) and extracted with chloroform (3 x 10mL). The organic extracts were combined, dried with magnesium sulfate, filtered and the solvent removed *in vacuo*. The resultant residue was then analysed by HPLC.

General Procedure for Horseradish Peroxidase

To a stirred solution of the quinone (0.6mmol) in acetonitrile (1.7mL) and phosphate buffer (0.1M, pH 6.0, 2.85mL) was added 0.45% hydrogen peroxide (0.75 eq., 3.3mL) and horseradish peroxidase (0.6mg, 200 units) at 35°C. Aliquots were removed at various time intervals. Ethyl acetate (0.5mL) was added to the aqueous solution and the solvent mixture was shaken vigorously. The organic layer was separated, dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The resultant residue was analysed by HPLC.

6.5 General Procedures for Chapter Five

- Human cervical cancer cells (HeLa cell line) were used for all biological experiments. The HeLa cells were cultured as monolayers in RPMI 1640 media with 10% Foetal Calf Serum, at 37°C with 5% CO_2. Progression of confluence was measured using an inverted light microscope. Cells were harvested using the following protocol: the existing media was decanted and the cells were washed twice with Phosphate Buffered Saline (PBS) prior to the addition of sufficient Trypsin-EDTA solution to cover the cell monolayer. The cells were then incubated for 5 min at 37°C. The cell suspension was then transferred to a centrifuge tube using 30mL of fresh media and spun at 800rpm for 3 min. The trypsin/media solution was decanted and the remaining pellet was resuspended in 5-10mL of fresh media. The cell density of the resulting suspension was then determined using a haemocytometer, which had a volume of 10^{-4}mL and was transposed with a 5 x 5 grid. The number of cells present in the 25 squares of the grid was counted using a light microscope (Olympus, BHB, 20x magnification) and the concentration of the cell suspension was thereby calculated.

- Effects on cell proliferation and viability were measured using a colorimetric MTT assay. To wells #2-11 of a 96 well flat-bottomed microtitre plate was added 50μL of fresh media (RPMI 1640 with 10% FCS). To well #1 was added 100 μL of a solution of the sample compound (of known concentration in media, made up from initial stock solution in Hybrimax DMSO) and this was serially diluted (1:1) down the plate, discarding after well #10. To wells #1–11 was added 50μL of cell suspension, such as to achieve a final concentration of

10,000 cells per well in 100μL for the assay. The cells were then incubated at 37°C for the appropriate period of time, after which the solution was tipped from the plate and the cells were washed with PBS (2 x 100μL). To each well was added 100μL of fresh media and 10μL of MTT (as a solution in PBS, $5mgmL^{-1}$), before incubation at 37°C for 5h. After this time, 100μL of solubilising agent (10% w/v sodium dodecylsulfate in 0.01M aqueous HCl) was added to each well of the plate before incubation overnight at 37°C. After this time, the absorbance of each well of the plate was read at 590nm by an ELISA automated spectrophotometric plate reader. Results were expressed as percentages of control (untreated cells) and were analysed using GraphPad Prism 2.0 software. All determinations were performed in quadruplicate and results were typical of multiple independent experiments.

- High performance liquid chromatography (HPLC) for Chapter Four was performed using an Agilent 1100 Series HPLC System, a Phenomenex Hypersil C18 reverse-phase column (250 by 4.6 mm) and a spectrometric detector operating at 290 nm with a flow rate of 1 mL/min. Two solvent systems were used: 1% trifluoroacetic acid in acetonitrile (C) and distilled water (D). The run started with 5% C and 95% D and was raised to 95% C and 5% D over 25 min, followed by isocratic elution for 5 min. The HPLC was coupled to a photodiode array detector for ultraviolet spectroscopic comparisons.

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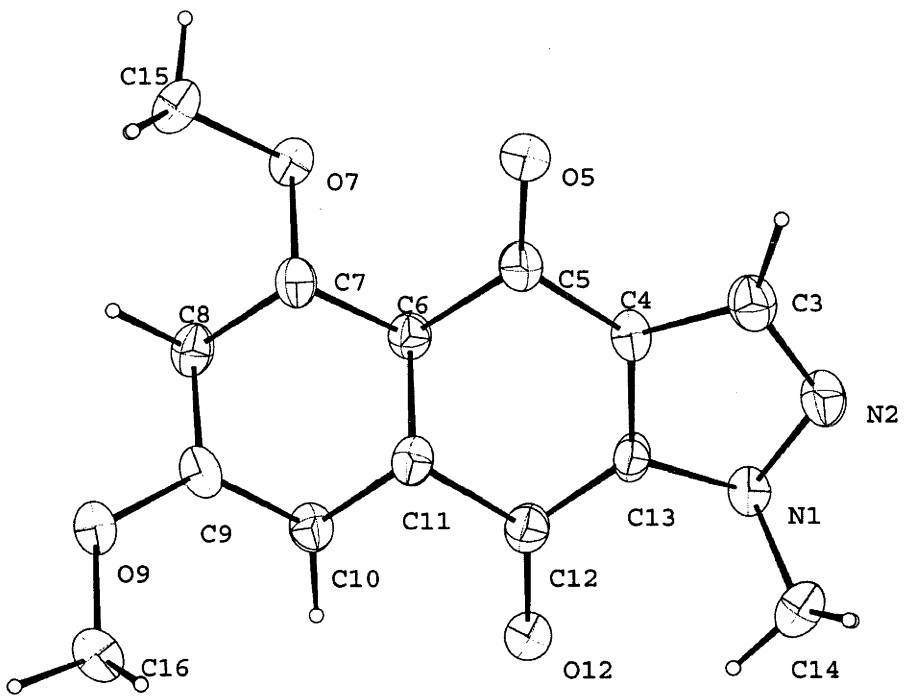
Appendices

Appendix A

*Crystal Data for 5,7-dimethoxy-1-methyl-1*H*-benzo[f]indazole-4,9-dione (2.30)*

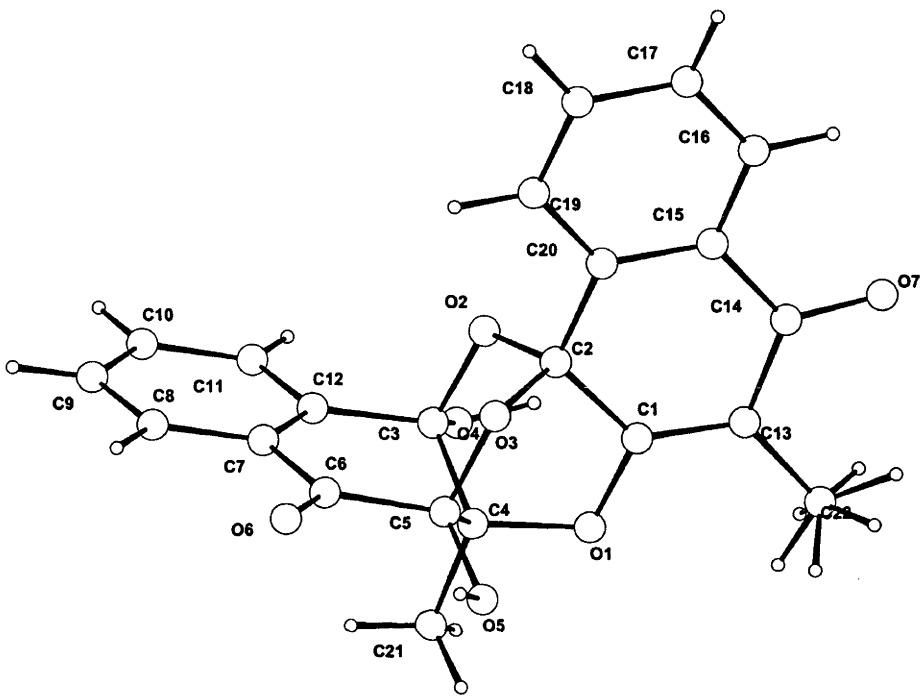


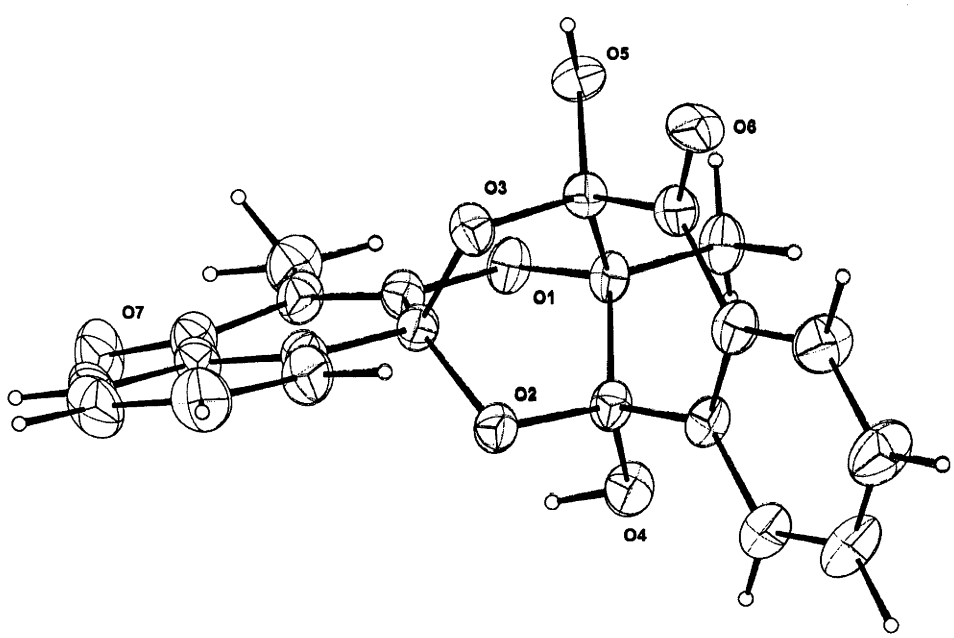
Empirical formula	$C_{14}H_{12}N_2O_4.CHCl_3$
Formula weight	391.638
Crystal Colour, Habit	Yellow, prism
Crystal Dimensions	0.293 x 0.292 x 0.194 mm
Crystal System	orthorhombic
No. of reflections used for Unit	
Cell Determination (2θ range)	33547 (2.910-25.028°)
Lattice Parameters	a = 6.5970 (2) Å

	b = 12.2276 (4) Å
	c = 20.9093 (6) Å
	V = 1686.66 (9) Å^3
Space Group	$P2_1ca$
Z value	4
D_{calc}	1.542 Mg m^{-3}
μ (CuKα)	0.56 mm^{-1}

Appendix B

Crystal Data for compound ***4.17***

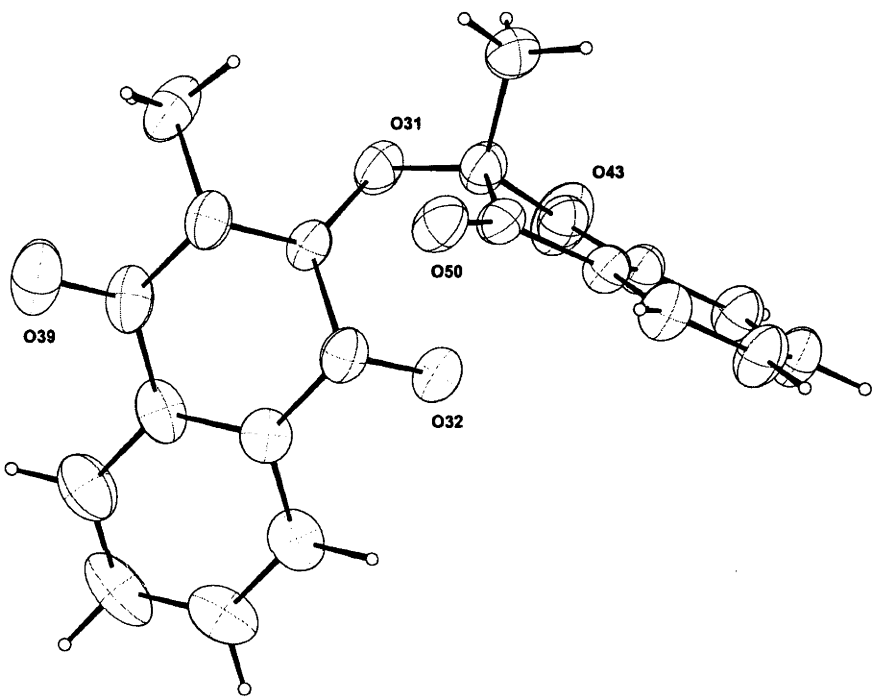




Empirical formula	$C_{22}H_{16}O_7.CHCl_3$
Formula weight	511.72
Crystal Colour, Habit	Colourless, prism
Crystal Dimensions	0.39 x 0.38 x 0.35 mm
Crystal System	Monoclinic
No. of reflections used for Unit	
Cell Determination (2θ range)	33931 (2.910-27.485°)
Lattice Parameters	a = 10.24380 (10) Å
	b = 11.59070 (10) Å
	c = 19.0555 (3) Å
	β = 90.9119 (5)°
	V =2262.23 (5) Å^3
Space Group	$P2_1/c$
Z value	4
D_{calc}	1.503 Mg m^{-3}
μ (CuKα)	0.45 mm^{-1}

Appendix C

*Crystal Data for 2-methyl-3-(2-methyl-1,3-dioxo-indan-2-yloxy)-1,4-naphthoquinone (**4.18**)*



Empirical formula	$C_{21}H_{14}O_5.0.5(C_4H_8O_2)$
Formula weight	346.32
Crystal Colour, Habit	Yellow, prism
Crystal Dimensions	0.3 x 0.24 x 0.210 mm
Crystal System	Monoclinic
No. of reflections used for Unit	
Cell Determination (2θ range)	59931 (2.910-27.485°)
Lattice Parameters	a = 19.0938 (2) Å
	b = 7.86280 (10) Å
	c = 25.9681 (4) Å

	$\beta = 91.2136\ (4)^{\circ}$
	$V = 3897.74\ (8)\ Å^3$
Space Group	P2/*c*
Z value	8
D_{calc}	1.255 Mg m^{-3}
μ (CuKα)	0.090 mm^{-1}